Copyright

by

Kevin Michael Christmas

2012

The Thesis Committee for Kevin Michael Christmas
Certifies that this is the approved version of the following thesis:

The dose dependent effects of polyphenol supplementation on inflammatory markers following eccentric exercise

APPROVED BY
SUPERVISING COMMITTEE:

Supervisor:

Edward F. Coyle

Matthew R. Brothers

The dose dependent effects of polyphenol supplementation on inflammatory markers following eccentric exercise

by

Kevin Michael Christmas, B.S.

Thesis

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science in Kinesiology

The University of Texas at Austin

May 2012

Acknowledgements

First, I would like to thank Dr. Coyle for his time and effort involved in advising me throughout this project. I cannot begin to describe how grateful I am to have worked under Dr. Coyle the past 2 and a half years. It has been an honor working under him in that time. I would also like to thank the previous work of Justin Trombold, which set the foundation for this research study, and his constant involvement with this study to ensure we performed it the best we could. I would also like to thank the other graduate students, Daniel Machin, Ting Heng Chou, Doug Van Pelt, and Sarah Hill whose assistance in the study was invaluable. I want to thank Dan Machin for taking control of the recruitment and doing the difficult task of organizing all the trials every week. Ting Heng Chou performed incredibly with the number of times he ran down the side of the stadium with subjects without hesitation. Doug Van Pelt was always there to keep me grounded and play devil's advocate to ensure things ran smoothly. I would like to thank Sarah Hill who was very helpful in running much of the blood data I am about to show and her vigorous work with handling paperwork. I want to thank Dr. Brothers for helping me as my second reader so late in the semester. I would also like to thank Mina Rathburn and Patty Coffman for all the support they provided behind the scenes of our study. I would like to additionally thank Dr. Trevor Gillum for helping me through every stage of the writing process and helping me continuously with statistics. Finally, I would like to extend my deepest appreciation to Dr. Suzanne Schneider who helped build me into the researcher I became and all of her help and support throughout this project.

ABSTRACT

The dose dependent effects of polyphenol supplementation on inflammatory markers following eccentric exercise

Kevin Michael Christmas, M.S.Kin

The University of Texas at Austin, 2012

Supervisor: Edward F. Coyle

Unaccustomed eccentric exercise can lead to decreases in muscle force production, increases in soreness, swelling, stiffness, and discomfort. The purpose of this study was to test the dose response of pomegranate juice concentrate on serum markers of muscle permeability, inflammation, and total anti-oxidant status. To test this hypothesis, 45 healthy recreationally active males (22.3 ± 0.5 y, 73.8 ± 1.71 kg, 174.9 ± 0.9 cm) were recruited from the local Austin community for participation in this study. Subjects were disqualified from participation in the study if in the past 6 months they were engaged in an exercise training program. Subjects were placed into either the placebo group, the once-daily, or twice-daily pomegranate juice concentrate supplementation group. Subjects performed a total of 8 days of supplementation. On day 4, all subjects came to the laboratory and underwent an eccentric exercise protocol consisting of 2 minutes of downhill running at -10% grade at 7.5 mph repeated 10 times, resulting in ~20 minutes of total downhill running. Thereafter, subjects performed 50 eccentric elbow extensions

each lasting 5 seconds using a weight equal to their concentric one-repetition maximum. Blood measures were made pre-exercise (baseline), and 2, 24, 48, 72, and 96 hours post exercise and analyzed for interleukin-6, creatine kinase, myoglobin, and total anti-oxidant status. Creatine kinase was significantly elevated at 96 hours post exercise, but with no significant differences between treatments. Myoglobin was significantly elevated above baseline at 2 and 96 hours, but with no differences between treatments. There was no effect for time or treatment on the total anti-oxidant status of the serum. Il-6 was significantly higher at 2 and 24 hours after exercise, but with no difference between treatments. The percent increase in interleukin-6 from baseline was significantly lower in the twice-daily POM supplementation group versus placebo (124.3 ± 9.4, 188.6 ± 16.0% of baseline; respectively) during the 2-96 hours following eccentric exercise, but no statistical difference between 1x and 2x or 1x and placebo was observed. This suggests that 8 days of supplementation with pomegranate juice concentrate twice a day significantly reduces the percent increase in a marker of inflammation (interleukin-6) during the 96 hours following eccentric exercise; however, neither supplement was different than the placebo in regards to all other measures.

Table of Contents

List of Tables

List of Figures

INTRODUCTION

Unaccustomed eccentric exercise results in decreased muscle force production, increased soreness, and prolonged recovery lasting longer than 24-96 hours. The nature of eccentric exercise and the elongation while muscle contraction takes place results in very high force production across the muscle tissue. This results in sarcomeres that are non-uniformly stretched beyond their optimum length and longer sarcomeres may experience rapid and forceful stretching that results in them 'popping' and becoming damaged [1]. This results in immediate strength loss due to mechanical damage and mediates an inflammatory process aimed at regeneration and repairing the muscle tissue.

The prolonged recovery could be a result of inflammation and oxidative stress following eccentric exercise [2-4]. Multiple studies have shown a relationship between muscle damage and inflammation and leukocyte infiltration suggesting a potential detrimental role of inflammation with eccentric exercise [5, 6]. The localization of immune cells can exacerbate damage to the muscle tissue due to macrophage phagocytosis generating free radicals, increasing oxidative stress, and aiding in muscle damage. It would therefore be advantageous to formulate interventions to attenuate this inflammatory response and possibly improve recovery following eccentric exercise.

Previous studies examining polyphenol supplementation have shown an ergogenic effect with eccentric exercise [7-9]. These studies showed improved strength recovery with supplementation following eccentric exercise. Unfortunately, none of these studies were able to identify a mechanism resulting in improved recovery. It was however

suggested that attenuation of the inflammatory process could be a potential mechanism [7]. Of greater importance is the fact that none of the studies that use polyphenols to improve muscle function and recovery following eccentric exercise have tried to find an optimal dosage for supplementation [8-12]. Therefore, we propose to determine the effects of supplementation with pomegranate juice concentrate once or twice daily on markers of inflammation following eccentric exercise. We hypothesize that supplementation twice a day will result in lower measures of inflammation following eccentric exercise than once a day supplementation or placebo. Our second hypothesis is that once a day supplementation will result in lower measures of inflammation following eccentric exercise than the placebo.

REVIEW OF LITERATURE

ECCENTRIC EXERCISE

When muscle contracts concentrically it is activated and shortens while producing force. Alternatively, an eccentric contraction occurs when muscle is lengthened while producing very high force. When the volume and intensity of eccentric contractions becomes unfamiliar or novel it can be associated with muscle damage [13-15]. Individuals who are unaccustomed to eccentric muscle damage will experience the most dramatic losses in strength, increases in stiffness, swelling, soreness, and discomfort in the days following eccentric exercise [13-15]. The etiology of eccentric muscle damage lies in the contraction of the muscle as it elongates instead of shortens, which generates high levels of tension across the muscle tissue resulting in tissue damage. This initial insult to the muscle causes immediate losses in strength, but also signals the localization of immune cells to remove and repair damaged tissue [14, 15]. This immune response helps to regenerate damaged tissue and may also be what causes the prolonged damage and delayed onset muscle soreness. It however has also been shown that this immune response may be beneficial for adaptation and confer protection against subsequent bouts of eccentric exercise. There have been attempts in the literature to attenuate the severity of the pro-inflammatory response and the delayed onset muscle soreness following eccentric exercise, but with equivocal findings.

INITIAL INSULT

Delayed onset muscle soreness can occur after unaccustomed exercise with high force generation, more specifically, when the exercise is eccentric in nature. Delayed onset muscle soreness is a type of muscle strain that occurs and creates stiffness and swelling following muscle insult. Eccentric exercise is unique in that while the muscle is activated the muscle elongates rather than shortens. This mode of exercise typically results in higher tension across the muscle body. The higher tension with elongation is thought to lead to the weaker portions of the muscle being damaged, which can be seen immediately post exercise by histologically examining the streaming of the contractile units [13, 14, 16]. This streaming has been hypothesized to be due to the 'popping' of sarcomeres [17]. This theory states that during lengthening of the muscle, sarcomeres are non-uniformly stretched beyond their optimum length and longer sarcomeres may experience rapid and forceful stretching that result in them 'popping' and becoming damaged. The weak sarcomeres don't appear uniformly in the muscle tissue, but result in focal lesions of damaged contractile units that stream and disrupt adjacent compartments such as the t-tubules. Studies that support this hypothesis show elongation of the muscle, which takes the sarcomeres out of their optimum length, results in more severe damage than when muscles are lengthened without taking sarcomeres out of their optimum length [18-20]. For example, Morgan et al. was able to show that the mere act of stepping down stairs two at a time with one leg while stepping down one at a time with the other leg resulted in subjects experiencing more pain and muscle damage with the leg that stepped down two stairs than the one that stepped down one stair at a time [1]. They suggested

that the nature of elongating the muscle by descending two steps at a time generates more weakened sarcomeres than when descending one stair at a time.

Disruption of the muscle tissue after mechanical stress results in an immediate loss in strength that can persist for several days [2, 21, 22]. This hallmark symptom of eccentric exercise is also considered to be one of the valid and reliable measures of muscle damage [23]. While soreness will peak 24-48 hours following eccentric exercise it is not a good reflection of muscle damage [2]. Concentric exercise can produce strength losses of 10-30%, but strength returns to baseline in the hours following exercise and is suggested to be due to fatigue rather than muscle damage [15, 24]. Alternatively, eccentric exercise can result in a 10-30% decrease in force production, with a recovery period of reduced strength lasting more than 96 hours [25-27], which is significantly longer than with concentric exercise [28]. The degree of strength loss is dependent upon the intensity of eccentric exercise and can reach as high as 50-65% loss in force production from baseline values [29, 30]. Different muscle groups may also experience different degrees of muscle damage. For example, downhill running protocols typically result in 20-30% decreases in leg strength [25, 31, 32], while maximal eccentric contractions of the elbow flexors have been reported to elicit a ~50% decrease in strength 24 hours following eccentric exercise [29, 30].

SECONDARY INSULT

The high degree of damage following eccentric damage occurs due to mechanical strain tearing the microstructure of the muscle [33]. In response to damage the body starts

producing inflammatory markers called cytokines. Cytokines are biological markers that facilitate the attraction of immune cells and can be considered chemo-attractant molecules. Following eccentric exercise the pro-inflammatory response that ensues can increase the intracellular and plasma concentrations of TNF-α, IL-1β, and interleukin-6 (IL-6), which signal for leukocyte infiltration. The accumulation of pro-inflammatory cell populations in the damaged tissue is likely to amplify the inflammatory injury to the muscle by the release of reactive oxygen species and with the activation of phospholipases and proteases in the tissue [34]. It is thought that the reactive oxygen species, phospholipases, and proteases facilitate the compromise of membrane permeability, calcium homeostasis, and can further degrade the muscle tissue following eccentric exercise [35].

Localization of immune cells within the muscle occurs within the first 24 hours following eccentric exercise [3, 36, 37] and has been shown to persist for as many as 14 days after eccentric exercise [38]. Neutrophils and macrophages exacerbate the muscle damage that occurs by generation of reactive oxygen species and nitrogen species [39, 40]. As the immune cells phagocytose the damaged particles, they generate reactive oxygen species that can damage surrounding tissue. The degree of secondary damage and infiltration of leukocytes that occurs following eccentric exercise is dependent upon the muscle groups recruited and severity of muscle damage inflicted [41]. The role of inflammation in the etiology of delayed onset muscle soreness and recovery is debatable. Some studies show the acute phase response is associated with delayed onset muscle soreness and loss in force production following eccentric exercise [5, 6], but other studies

suggest a beneficial role of inflammation in adaptation [42-44]. Research in mice suggests an association between muscle hypertrophy and Il-6 providing a potential adaptive role of Il-6 in eccentric exercise [43, 45]. It is however unknown how recovery is effected by inflammation and if attenuation of inflammation would be beneficial to the recovery process.

The training adaptations from repeated eccentric exercise may begin as early as within the first 24 hours after eccentric exercise [46]. Koh et al. was able to show, in a very eloquent design that the protection conferred from eccentric exercise can be attained by passive stretching of the muscle tissue [42]. Mice underwent passive elongation of the extensor digitorium longus muscle without signs of muscle injury or regeneration. The mice that underwent passive stretching experienced only a 36% decrease in force following eccentric exercise while control mice experienced a 55% loss. Pizza et. al added to this finding, utilizing the same model, and showing leukocyte infiltration following passive elongation [44]. Passive stretching had a leukocyte response roughly half of the magnitude of the control mice after eccentric exercise. The passive elongation group also experienced a blunted inflammatory response and leukocyte infiltration after performing eccentric exercise. These two studies suggest that in the absence of muscle damage or repair there exists a mechanism facilitating adaptation against eccentric exercise. Pizza et. al were able to show an acute phase response that could suggest a role of inflammation and leukocytes in facilitating said adaptation. However no assessments of oxidative stress were made and the role of free radicals in this process is unknown.

Reactive oxygen species are generated because of oxygen's diatomic nature. Oxygen found inside the body has two unpaired electrons in its outer shell, which can only be reduced one at a time resulting in the generation of free radicals [47]. Reactive oxygen species function by taking electrons from other molecules creating a new free radical in its place that results in a radical chain, which is typically terminated by reacting with an anti-oxidant [48].

Reactive oxygen species are capable of reacting with all major types of biomolecules, but lipids are particularly prone to oxidation [49, 50]. The fact that lipids are prone to oxidation has been one of the reasons that many oxidative stress markers use oxidized lipids. The nature of reactive oxygen species and their attraction towards lipid oxidation results in membranes being attacked frequently [48]. Because of radical chains and since free radicals can move from one lipid to a neighboring lipid, muscle damage can occur at the site of origin or elsewhere quite easily [51]. The source of reactive oxygen species following eccentric exercise is likely from phagocytes rather than from the electron transport chain or xanthine oxidase found in endothelial cells. Generation of reactive oxygen species from the electron transport chain is associated with aerobic metabolism and is an unlikely source during eccentric exercise because of the low metabolic cost involved compared to concentric exercise [15]. A noteworthy point is that certain immune cells are able to generate large quantities of reactive oxygen species, namely monocytes/macrophages, eosinophils, and neutrophils [52].

REPEATED BOUT EFFECT

Performing a single bout of eccentric exercise generates muscular adaptations that grant protection from further bouts of eccentric exercise. The degree of soreness and strength lost following each bout of eccentric exercise will be less severe than the previous bout [16, 25, 27, 53]. Adaptation to eccentric exercise has been shown to occur as early as within the first 24 hours following eccentric exercise [46]. Several other studies were able to show attenuations in creatine kinase (CK), soreness, and strength loss with repeated bouts of eccentric exercise [25, 46, 54]. Chen et al. was able to show that high intensity eccentric exercise provided greater protection than low intensity exercise with repeated bouts of eccentric exercise [55]. This would suggest a beneficial role for performing maximal eccentric contraction to maximize adaptations. However, Chen et al. showed in a later study that four low intensity bouts of eccentric exercise conferred as much protection as one bout of eccentric exercise at 100% of maximal voluntary contraction [56]. This could suggest more frequent less intense bouts of eccentric exercise could be less uncomfortable and still confer adequate protection. It might also suggest that screening of subjects needs to carefully take into account all forms of eccentric activity in daily living regardless of intensity before undergoing eccentric exercise. Rat studies have also shown that passive elongation of the muscle produces increases in interleukin-6 and infiltration of leukocytes, but also conferred protection against a subsequent bout of eccentric exercise in the absence of muscle damage or strength loss [42, 44]. This novel concept has even led to findings that suggest

undergoing static stretching or proprioceptive neuromuscular facilitation in humans attenuates the damage from a bout of eccentric exercise [57].

SUPPLEMENTATION

The effects of supplementation on eccentric exercise have led to controversy on their role to attenuate muscle damage and improve recovery [7, 9, 31, 58-66]. Previous studies have shown that anti-oxidant and anti-inflammatory supplementation can attenuate strength losses, inflammation, and muscle damage following eccentric exercise [7, 61, 65], but has also had equivocal findings as well [66-68]. This discrepancy in the literature is likely to stem from the supplement type.

Vitamin C & E supplementation together or separatly has mostly shown no beneficial effect following eccentric exercise [63, 68-73]. Vitamin C supplementation was thought to mediate the oxidative stress following eccentric exercise, but many studies show no effect of vitamin C on muscle damage [66, 68, 74, 75]. Regardless of dose or duration there appears to be no positive effect of vitatmin C supplementation. Vitamin E was thought to be a potentially good supplement due to its fat soluble nature and would therefore stay in the body longer and possibly interact with cellular membranes. Vitamin E supplementation has led to equivocal findings as several studies show no effect on muscle damage [70, 76], with some showing a reduction in muscle damage only in the young compared to the elderly [77-79]. The combination of vitamin E & C results in similar findings as most studies show no effect of supplementation on muscle damage [80, 81].

Interestingly, Phillips *et al.* was able to show the combination of vitamin E, omega-3 fatty acids, and flavanoids for 7 days was able to reduce markers of inflammation and oxidative stress 3 days after eccentric elbow flexion exercise [62]. In line with this literature, Taribian *et al.* showed lower TNF-α immediately, 24, and 48 hours following eccentric exercise [58]. They were also able to show lower elevation of Il-6, CK, and myoglobin (Mb). These two studies might suggest a role of omega-3 fatty acids, which are a known anti-inflammatory. Furthermore, curcumin supplementation, a known anti-inflammatory, in mice has been shown to reduce inflammation and improve muscle function following downhill running [61]. These studies suggest a potential role for dietary anti-inflammatories in reducing inflammation and muscle damage following eccentric exercise.

Polyphenols, such as ellagitannin, are commonly found in pomegranate juice and have anti-inflammatory and anti-oxidant properties. Polyphenols supplementation has led to positive findings and less equivocal responses following eccentric exercise [8, 9, 82]. Clinical applications for phenolics have also led to beneficial responses [83-85]. Tart cherry juice supplementation 4 days before and after an eccentric bout of exercise resulted in significantly higher strength at 24, 48, 72, and 96 hours post exercise compared to placebo [7]. Connolly et. al. suggested a potential role of reduced inflammation; however, these measures were not made. In addition tart cherry juice has been shown to reduce pain after a long distance running race [12], and was able to reduce oxidative stress (TBARS), inflammation (IL-6), and aid in recovery following a marathon [11]. Pomegranate juice has also had equally positive findings showing improved

recovery of strength following eccentric exercise [8, 9]. Despite the number of studies that are able to show beneficial effects of polyphenols supplementation on strength recovery few studies are able to show attenuations in pro-inflammatory cytokines, CK, and Mb [58].

CONTENTS OF POMEGRANATE JUICE

Polyphenols are typically found in the skin of brightly colored fruits or vegetables such as: tea, red wine, apples, pomegranates, cherries, coffee, blue berries, strawberries, and broccoli. Pomegranate juice has been shown to be beneficial by triggering apoptosis in cancer cells by modulating cell inflammatory processes [83]. It has also been shown to reduce the degree of reactive oxygen species released by macrophages [83, 84]. It has been suggested that compounds found in pomegranate juice work together synergistically granting it potent anti-oxidant capabilities [85]. In line with this finding, supplementation in mice has shown to be effective in reducing systemic oxidation of the liver [86]. Trombold et al. was able to show improved recovery of strength following eccentric exercise in two separate studies [8, 9], but was unable to elaborate on a mechanism as there was no effect on the blood measures of damage and inflamation [9]. Pomegranate juice is a potent anti-inflammatory and anti-oxidant due to the high concentrations of polyphenols, which include ellagitannins, ellagic acid, quercitine, kaempferol, and luteolin glycosides [87]. Of these the most prevalent is pinicalagin, an ellagitannin, which is considered to be responsible for over half of pomegranate juice’s anti-oxidant activity [88].

BLOOD MARKERS OF MUSCLE DAMAGE

Blood markers of muscle damage are commonly measured with eccentric exercise and include: CK, Mb, lactate dehydrogenase, and myosin heavy-chain fragments. These markers however correlate poorly with muscle function and may not represent muscle damage per se [26, 89]. Furthermore, repeated bouts of eccentric exercise can result in an elimination of CK leakage into the blood while still experiencing disruption of the contractile units [27]. Creatine kinase measurements are based on activity not concentration and may not fully represent the flux of CK out of the damaged tissue. Creatine kinase has sulfhydryl (-SH) bonds, which are prone to oxidation and inactivation of the enzyme [90, 91]. It also is known that CK activity following eccentric exercise varies, likely due to oxidation and inactivation of the enzyme [91]. These findings suggest Mb as a better measure of damage with eccentric exercise, but it should be noted that CK and Mb are indices of membrane permeability and are poor reflections of muscle damage [23, 26, 89].

Inflammatory markers measured after eccentric exercise is typically in the form of cytokines, chemokines, or clinical markers like C-reactive protein [8, 61, 62, 92, 93]. The assessment of inflammatory markers can be done locally through muscle biopsies or isolation of muscle in animal models or systemically through blood markers. More studies assess inflammation with systemic measures because the invasive nature of a muscle biopsy has been shown to increase inflammatory markers and cause leukocyte infiltration in the absence of eccentric exercise [94].

Oxidative stress is often measured by analyzing the oxidation of bimolecular markers after exercise. Typical markers are reduced glutathione, t-bars, plasma isoprostanes, or ferric reducing activity of plasma [95-98]. Additional measures are total anti-oxidant status of the blood, which assesses all endogenous and exogenous anti-oxidants ability to reduce free radicals. This measure is more physiologically relevant as it doesn't measure any one anti-oxidant [73, 99].

METHODS

SUBJECTS

Forty-five healthy, non-smoking, males (22.3 ± 0.6 y, 73.8 ± 1.71 kg, 174.9 ± 0.92 cm) were recruited from the local Austin community for participation in this study. Subjects were disqualified from participation in the study if in the past 6 months they were engaged in an exercise training program or sustained an injury to the arms or legs, or participated in any physical therapy. Subjects were asked to refrain from the consumption of vitamin, mineral, anti-inflammatory or anti-oxidant supplements, and any over the counter medications throughout the duration of the testing period. Additional exclusion criteria was having a history of hypertension, active weight loss > 5 kg in the past 3 months, and having any history of kidney dysfunction. They were also asked to discontinue any type of exercise outside of the study throughout the testing period. This study was conducted with the approval of the University of Texas Institutional Review Board, and each subject provided written consent.

DESIGN

This study was a double blind, counter balanced, placebo controlled experiment with an 8-day supplementation period. Subjects took a one oz supplement of pomegranate juice concentrate or placebo, twice daily, approximately at 7 am and 7 pm. Subjects in the placebo group consumed placebo in the morning and the evening. Subjects in the 1x group took pomegranate juice concentrate in the morning and placebo in the evening, while the 2x group consumed pomegranate juice concentrate both in the morning and evening. On the morning of the 4^{th} day of supplementation subjects performed a bout of

eccentric exercise used to elicit delayed onset muscle soreness. Subjects were assigned using a counter-balanced design into one of three groups: placebo (PLA), once-daily (1x), or twice-daily (2x) pomegranate juice supplementation.

Blood samples were taken before supplementation on day 1 (pre), before eccentric exercise on day 4 (baseline), 2 hours, 24 hours, 48 hours, 72 hours, and 96 hours after the eccentric exercise. All blood draws were taken at the same time of day in the morning with subjects in a similar postprandial state.

SUPPLEMENTATION

Subjects took one ounce of placebo or pomegranate juice concentrate diluted with water to taste, which was taken at approximately 7 a.m. and 7 p.m. throughout the 8 day supplementation period. The 1x group took the pomegranate juice concentrate in the morning and placebo in the evening to ensure all groups still took supplements twice a day. The 2x group took pomegranate juice concentrate both in the morning and evening. The placebo group took a placebo both in the morning and the evening. All supplements were provided by POM Wonderful (Los Angeles, CA). Each dose of pomegranate juice concentrate, one oz, contained 650 mg gallic acid equivalents (GAE) per dose. The pomegranate concentrate contained 62g of carbohydrate per 1 oz (total sugars: 49g, glucose: 25g, fructose: 24g), while the placebo contained 65g of carbohydrate per 1 oz (total sugars: 63g, glucose: 35g, fructose: 28g). The placebo was matched by color, flavor, and carbohydrate content to the pomegranate concentrate supplement as best as possible.

ECCENTRIC EXERCISE

Subjects performed an intermittent downhill running protocol, which consisted of 2 minutes of downhill running at -10% grade at approximately 7.5 mph repeated 10 times and resulting in roughly 20 minutes of total running. This was performed by running down the ten story concrete ramps of the football stadium. Time between repetitions lasted roughly 2-4 minutes as subjects rode the elevator back up to the 11^{th} floor. After completion of the downhill running portion of the eccentric exercise subjects performed 50 eccentric elbow extensions (i.e.: preacher curls) each lasting 5 seconds using a weight equal to their concentric one-repetition maximum. The one-repetition maximum was determined by finding the highest weight the subject could lift once by continuing to add weight to a curling bar until the subject failed to complete a repetition. The subjects' one-repetition maximum was determined as the highest weight they could lift only once.

Blood Collection

Blood was collected from the anticubital vein and placed into serum separating tubes (Vacutainer, Franklin Lakes, NJ). The blood was left at room temperature for 45 minutes to coagulate, which was then centrifuged at 1500 G for 10 minutes at 4˚C and aliquoted into empty micro-centrifuge tubes. All samples were stored immediately at -80˚C.

SERUM ANALYSIS

Serum was analyzed for creatine kinase, myoglobin, total anti-oxidant status, and interleukin-6 using commercially available assays (Pointe Scientific, inc; Oxis International, Foster City, CA; Caymen Chemical, Ann Arobor, MI; Enzo Life Sciences, Farmingdale, NY). Samples were centrifuged at 17000 G for 5 minutes at 4˚C before

analysis. The intra-assay and inter-assay reliability, as determined by the manufacturer, for creatine kinase was 0.65%, 0.75%; myoglobin was 5.44%, 8.25%; total anti-oxidant status was 3.4%, 3.0%;, and interleukin-6 was 5.3%, 6.5%; respectively.

CREATINE KINASE

Creatine kinase was measured in duplicate in the serum by measuring the enzyme kinetics of creatine kinase for converting ADP into ATP and eventually generating NAD^+. Samples were measured by placing a small volume of sample, ~25 µl, into 1.0 mL of cocktail and watching appearance of NAD^+ by reading at 340 nm on a spectrophotometer. By measuring the rate of appearance and utilizing the Beer-Lambert law creatine kinase was quantified. As this assay is dependent upon enzyme kinetics the values represent activity of creatine kinase in the serum rather than a concentration.

MYOGLOBIN

Myoglobin concentration was measured in duplicate in the serum using the enzyme-linked immunosorbent method. Samples of serum were pippetted into antibody coated wells that bind Mb. A secondary antibody is added, which binds to the myoglobin, which contains an enzyme subunit generating a color change when the final photosensitive chemicals are added. The assay is measured using a spectrophotometer plate reader at 450 nm.

TOTAL ANTI-OXIDANT STATUS

Total anti-oxidant status was measured in duplicate in the serum by utilizing a colorometric method whereby hydrogen peroxide oxidizes trolox resulting in a color

change. The assay measures all of the anti-oxidants in the sample by measuring how well the serum can prevent oxidation of trolox. Samples are quantified using a mM trolox equivalent standard as opposed to concentration of individual anti-oxidants in the serum.

INTERLUEKIN-6

Interluekin-6 was measured in duplicate using the enzyme-linked immunosorbent method. Samples of serum were pippetted into antibody coated wells that bind Mb. A secondary antibody is added, which binds to the myoglobin, which contains an enzyme subunit generating a color change when the final photosensitive chemicals are added. This assay was unique in that samples had a blank sample that was subtracted from each sample's optical density. This step doesn't occur in all assays and is typically added at the manufacturer's discretion. This step occurs to help remove some of the back ground noise.

STATISTICAL ANALYSIS

Means and standard error were calculated for each of the descriptive statistics. A two-way analysis of variance was used to compare creatine kinase, myoglobin, total anti-oxidant status, and IL-6 between groups and across time. Significance was set at an alpha of 0.05. A Tukey's post-hoc analysis was used when significance was reached. Statistics were analyzed using SPSS (IBM, Armonk, NY).

RESULTS

EXERCISE

All three groups performed 10 repetitions of downhill running with -10% grade with no difference in speed (PLA: 7.73 ± 0.04, 1x: 7.61 ± 0.05, 2x: 7.64 ± 0.04 miles per hour; respectively) measured by knowing the distance traveled and the time it took to descend 10 flights of ramps. Subjects performed 50 eccentric elbow extensions (i.e.: preacher curls) at their one-repetition maximum (PLA: 30.61 ± 1.97, 1x: 31.82 ± 2.13, 2x: 30.61 ± 1.97 kilograms; respectively; $p > 0.05$) with no difference between groups in weight lowered (PLA: 1224 ± 97, 1x: 1139 ± 105, 2x: 1224 ± 97 kilograms; respectively).

CREATINE KINASE

There was a main effect of time ($p < 0.01$) for CK. A Tukey's post-hoc test revealed that 96 hours post exercise was significantly higher than baseline, 2, 24, 48 hours post exercise (1878 ± 744, 163 ± 19, 231 ± 18, 373.2 ± 30, 417 ± 112 U/L; respectively; $p < 0.04$) (Figure 1).There was no difference between PLA, 1x, and 2x (476.2 ± 94.5, 567.2 ± 163.0, 1001 ± 388.6 U/L; respectively) (Figure 2). Creatine kinase was significantly correlated with myoglobin ($r = 0.63$, $p < 0.01$) (Figure 3). Outliers were removed and creatine kinase was again significantly correlated with Mb ($r = .195$, $p < 0.01$) (Figure 4). Outliers were deemed as individuals that exhibited values ~4 times the group mean at any time point for either creatine kinase or myoglobin.

MYOGLOBIN

There was a main effect for time ($p < 0.01$) for myoglobin and Tukey's post-hoc test revealed that 2 and 96 hours post exercise were significantly greater than baseline (148±

14, 152 ± 44, 22 ± 2 ng/mL; respectively; $p < 0.03$) (Figure 5). There was no difference between PLA, 1x, and 2x (68 ± 9, 94 ± 16, 121 ± 30 ng/mL; respectively) (Figure 6).

TOTAL ANTI-OXIDANT STATUS

There was no main effect for time (Pre: 1.50 ± 0.07; Baseline: 1.48 ± 0.07, 24: 1.62 ± 0.06 mM Trolox equivalents; respectively; $p > 0.05$) (Figure 7). There was no main effect for treatment (PLA: 1.51 ± 0.07, 1x: 1.53 ± 0.07, 2x: 1.55 ± 0.07 mM Trolox equivalents; respectively) (Figure 8).

INTERLUEKIN-6

There was a main effect for time ($p < 0.001$). A Tukey's post-hoc test revealed that 2 and 24 hours post exercise were statistically higher than baseline, 48, 72, and 96 hours post exercise (5.40 ± 0.29, 5.20 ± 0.47, 2.87 ± 0.17, 2.80 ± 0.27, 3.18 ± 0.30, 2.89 ± 0.30 pg/mL; respectively; $p < 0.01$) (Figure 9). There was no main effect between treatment groups (PLA: 3.72 ± 0.22, 1x: 3.90 ± 0.46, 2x: 3.82 ± 0.24 pg/mL; respectively; $p > 0.05$) (Figure 10). When the data was described as the percent increase in IL-6 over baseline there was a main effect for time ($p < 0.001$) and for treatment ($P < 0.002$). Tukey's post-hoc test revealed that 2 hours post exercise was significantly different from 0, 48, 72, and 96 hours post exercise(228.15 ± 21.40, 100 ± 0.00, 106.16 ± 11.74, 131.58 ± 16.69, 119.79 ± 15.84% higher than baseline; respectively; $p < 0.002$) (Figure 11). Tukey's post-hoc analysis also revealed that 24 hours post exercise was significantly different from 0, 48, and 96 hours post exercise (195 ± 19.43, 100 ± 0.00, 106.16 ± 11.74, 119.79 ± 15.84% higher than baseline; respectively; $p < 0.05$) (Figure 11). Tukey's post-hoc test revealed that the 2x group, as a treatment, was significantly lower than PLA (124.35 ±

9.46, 188.68 ± 16.01% of baseline; respectively; $p < 0.001$) during the 2-96 hour period (Figure 12).

Figure and Table Legends:

Figure 1: Serum creatine kinase concentration *vs.* time. * Baseline (0), 2, 24, 48 hours post exercise were significantly lower than 96 hours post exercise ($p < 0.04$).

Figure 2: Serum creatine kinase concentration *vs.* time with comparison between treatments. There was no difference between treatment groups.

Figure 3: Serum creatine kinase concentration *vs.* serum myoglobin concentration and was significantly correlated ($r = 0.63$; $p < 0.01$).

Figure 4: Serum creatine kinase concentration *vs.* serum myoglobin concentration, without outliers, was significantly correlated ($r = .195$, $p < 0.01$). Outliers were defined as individuals that exhibited values ~4 times the group mean at any time point for either creatine kinase or myoglobin and were removed from the above analysis.

Figure 5: Serum myoglobin concentration *vs.* time. * 2 and 96 hours post exercise are significantly higher than baseline (0 h) ($p < 0.03$).

Figure 6: Serum myoglobin concentration *vs.* time with comparison between treatments. There was no difference between treatment groups.

Figure 7: Total anti-oxidant status *vs.* time. There was no difference across time.

Figure 8: Total anti-oxidant status *vs.* time with comparison between treatments. There was no difference between treatment groups.

Figure 9: Serum Interluekin-6 concentration expressed in picograms per milliliter (pg/mL). * 2 and 24 hours post exercise are significantly higher than baseline (0), 48, 72, and 96 hours post exercise ($p < 0.01$).

Figure 10: Serum Interluekin-6 concentration vs. time with comparison between treatments. There was no difference between treatment groups.

Figure 11: Serum Interluekin-6 concentration expressed as a percentage of baseline (0). * 2 hours post exercise is significantly different from baseline (0), 48, 72, and 96 hours post exercise ($p < 0.001$). † 24 hours post exercise is significantly different from baseline (0), 48, and 96 hours post exercise ($p < 0.01$).

Figure 12: Serum Interluekin-6 concentration expressed as a percentage of baseline. * PLA is significantly higher than 2x during the 2 to 96 hours post exercise ($p < 0.001$).

Figure 1

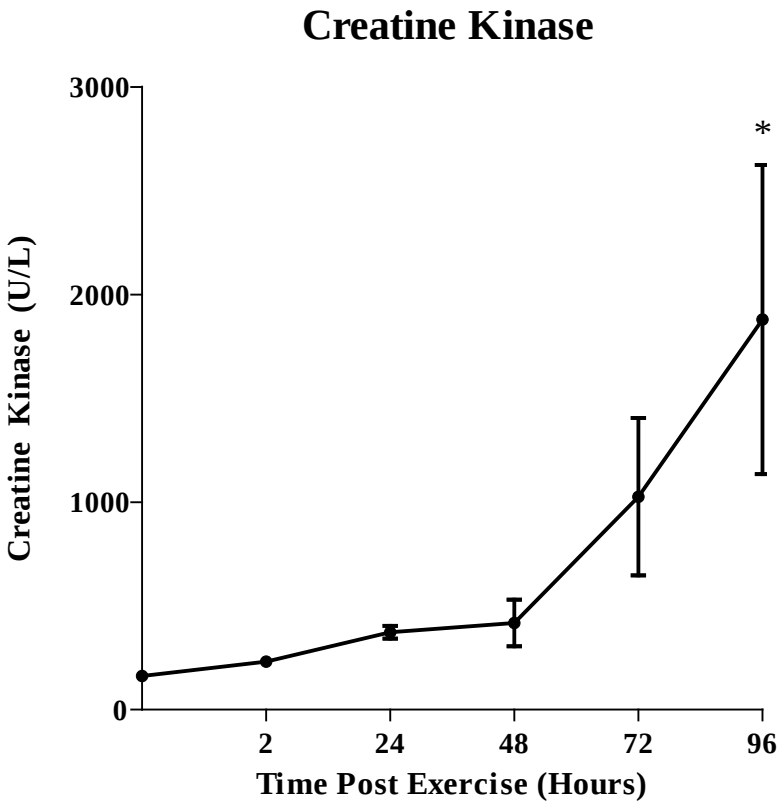

Creatine Kinase
3000
2000
1000
0
Creatine Kinase (U/L)
*
2
24
48
72
96
Time Post Exercise (Hours)

Figure 2

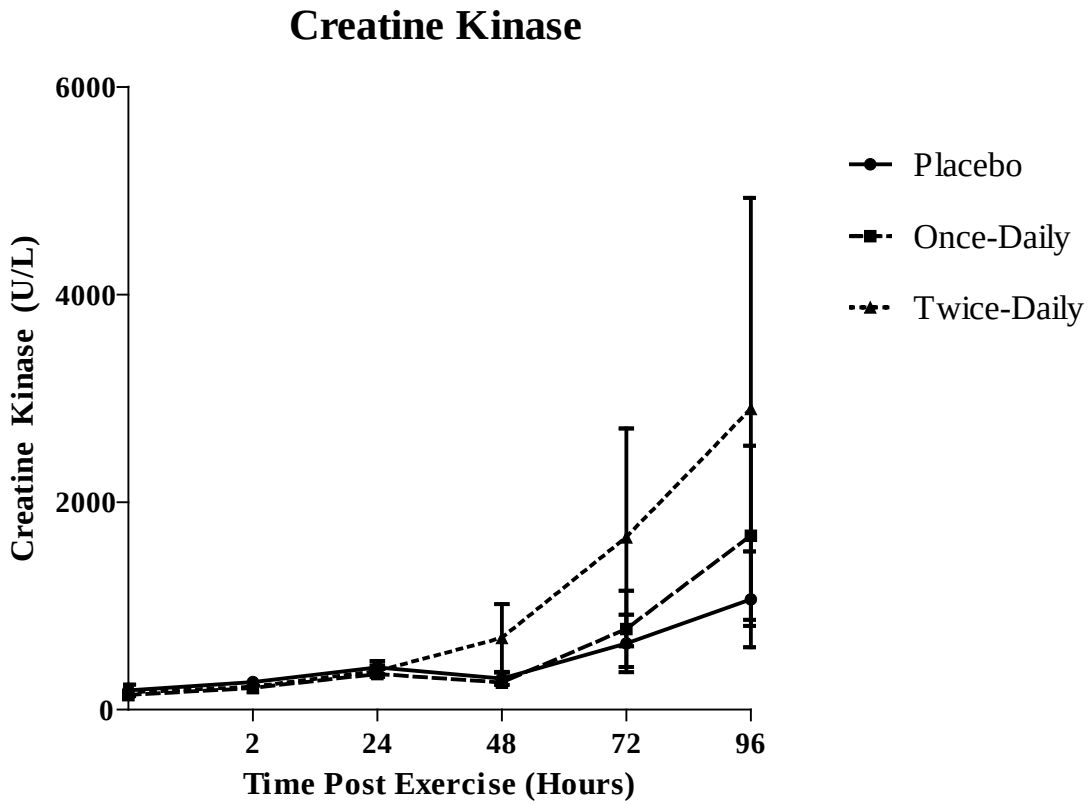

Creatine Kinase
6000
4000
2000
0
Creatine Kinase (U/L)
2
24
48
72
96
Time Post Exercise (Hours)
Placebo
Once-Daily
Twice-Daily

Figure 3

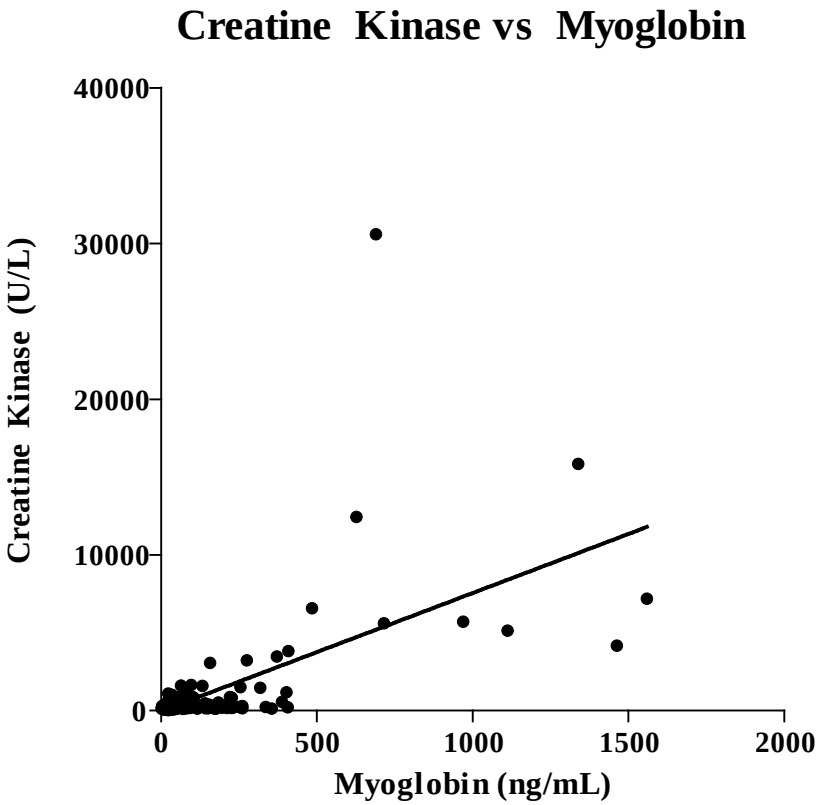

Creatine Kinase vs Myoglobin
Creatine Kinase (U/L)
40000
30000
20000
10000
0
0
500
1000
1500
2000
Myoglobin (ng/mL)

Figure 4

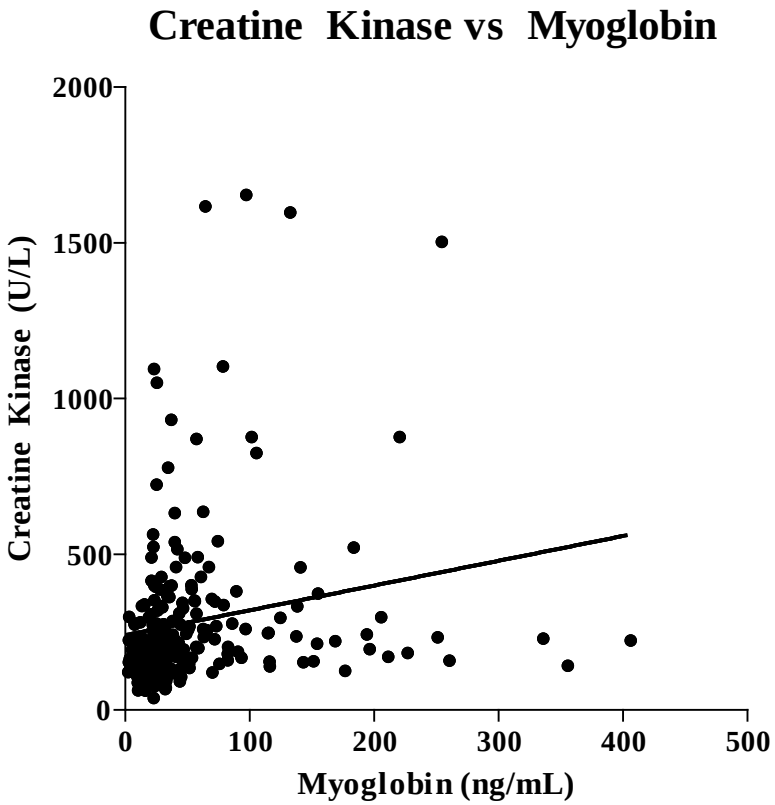

Creatine Kinase vs Myoglobin
Creatine Kinase (U/L)
2000
1500
1000
500
0
0
100
200
300
400
500
Myoglobin (ng/mL)

Figure 5

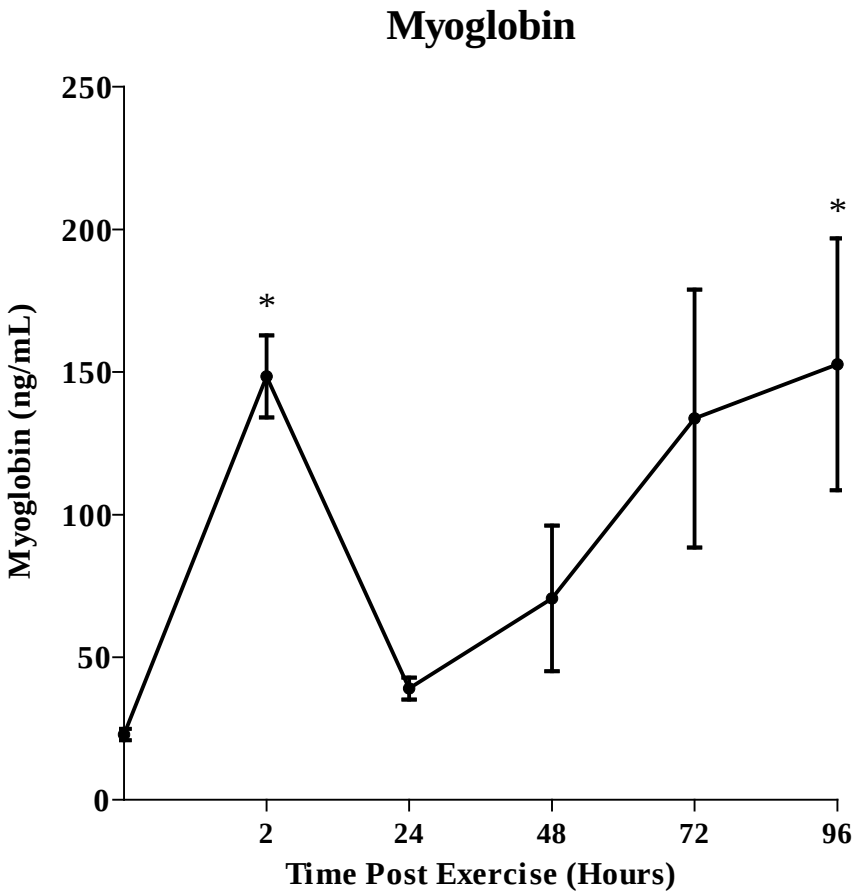

Myoglobin
250
200
150
100
50
0
Myoglobin (ng/mL)
*
*
2
24
48
72
96
Time Post Exercise (Hours)

Figure 6

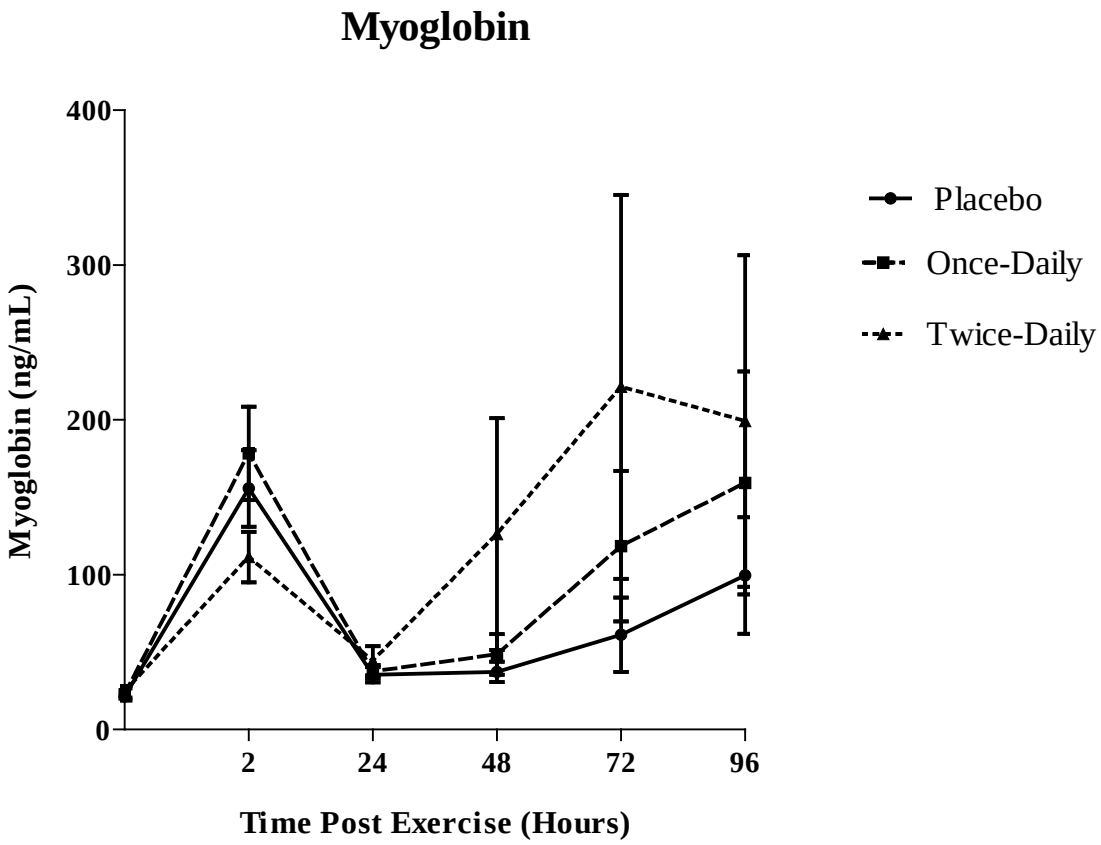

Myoglobin
400
300
200
100
0
Myoglobin (ng/mL)
2
24
48
72
96
Time Post Exercise (Hours)
Placebo
Once-Daily
Twice-Daily

Figure 7

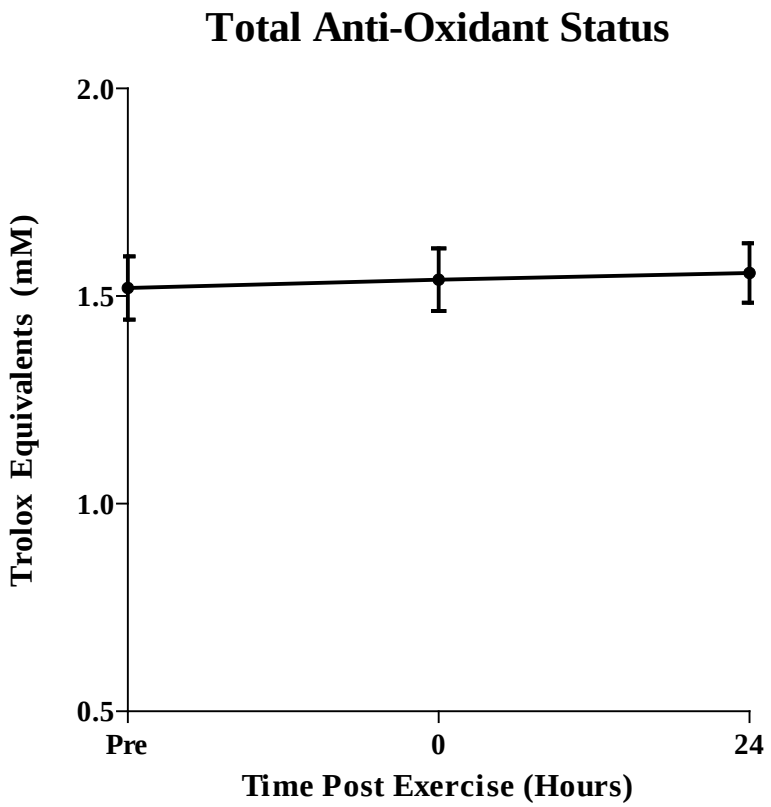

Total Anti-Oxidant Status
Trolox Equivalents (mM)
2.0
1.5
1.0
0.5
Pre
0
24
Time Post Exercise (Hours)

Figure 8

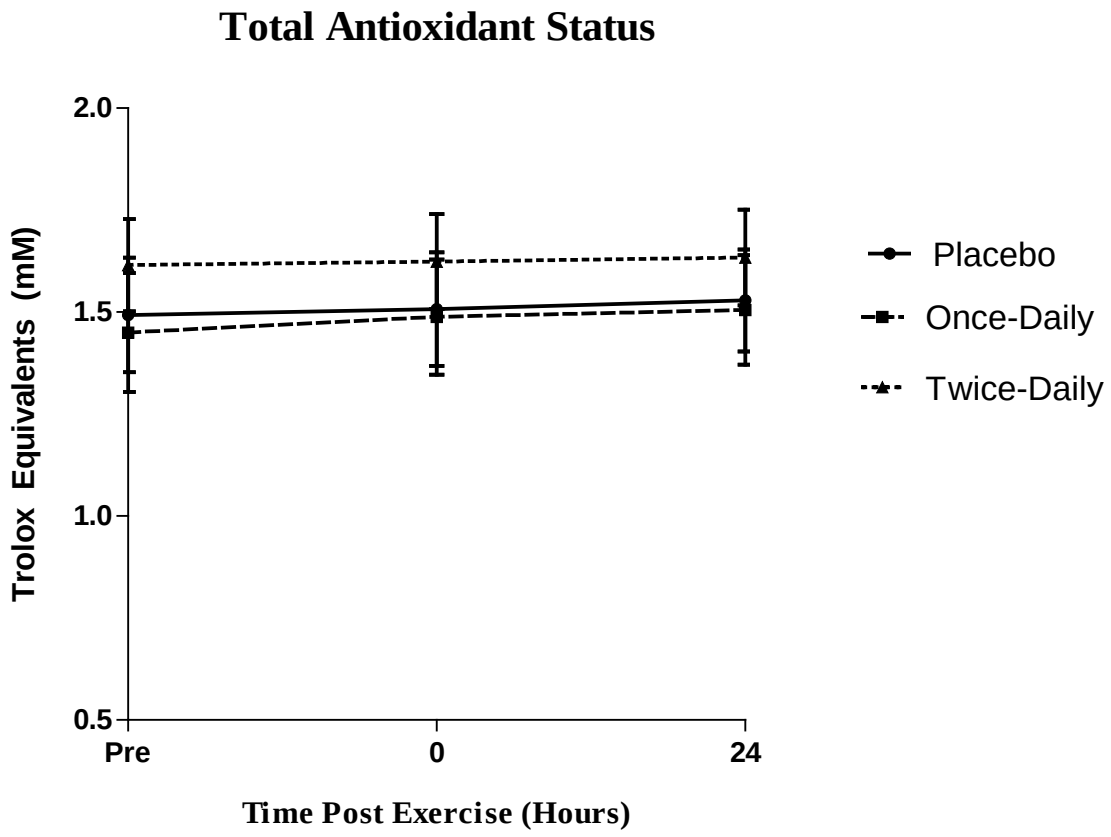

Total Antioxidant Status
2.0
1.5
1.0
0.5
Trolox Equivalents (mM)
Pre
0
24
Time Post Exercise (Hours)
Placebo
Once-Daily
Twice-Daily

Figure 9

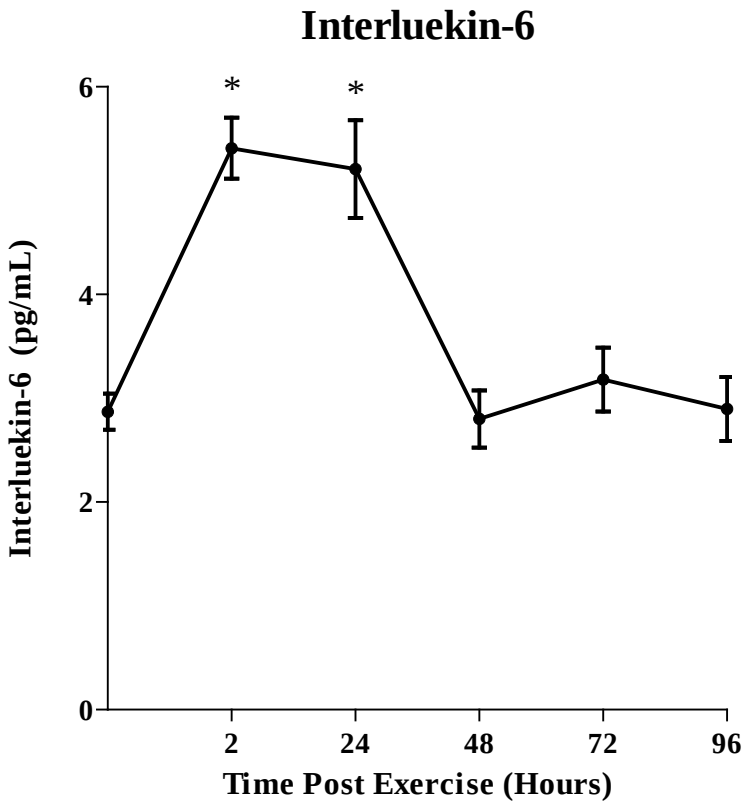

Interluekin-6
Interluekin-6 (pg/mL)
6
4
2
0
*
*
2
24
48
72
96
Time Post Exercise (Hours)

Figure 10

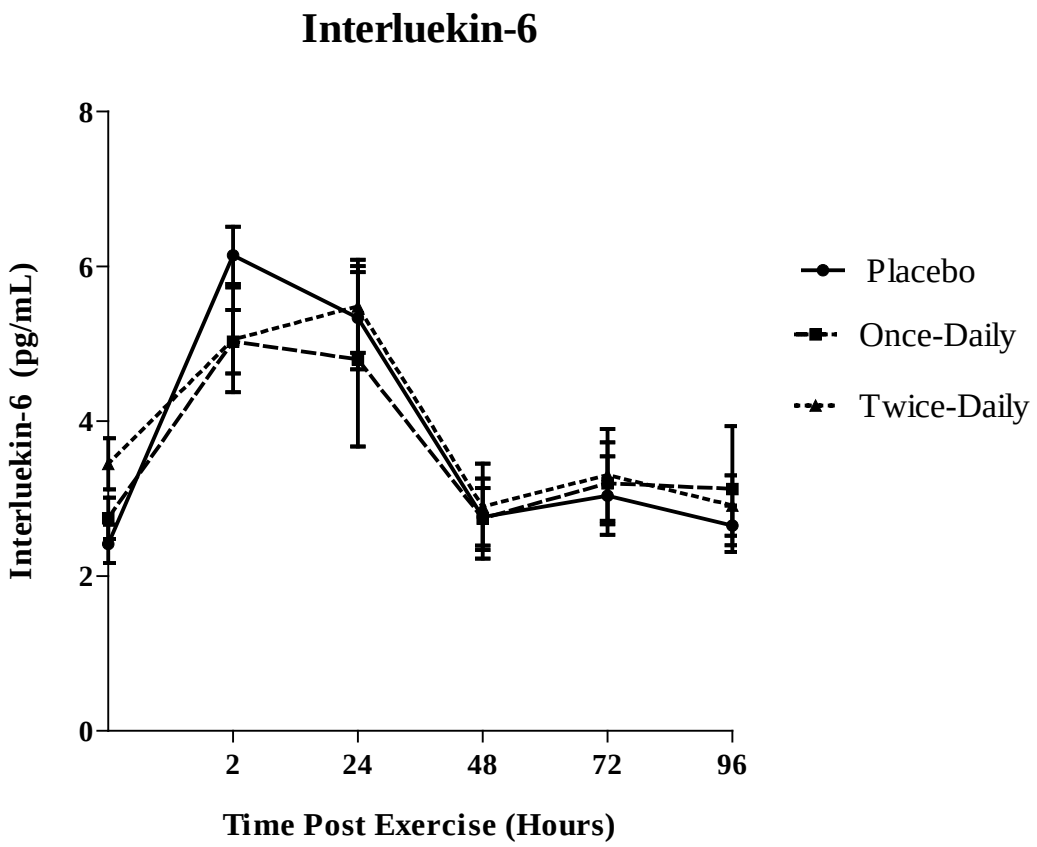

Interluekin-6
Interluekin-6 (pg/mL)
8
6
4
2
0
2
24
48
72
96
Time Post Exercise (Hours)
Placebo
Once-Daily
Twice-Daily

Figure 11

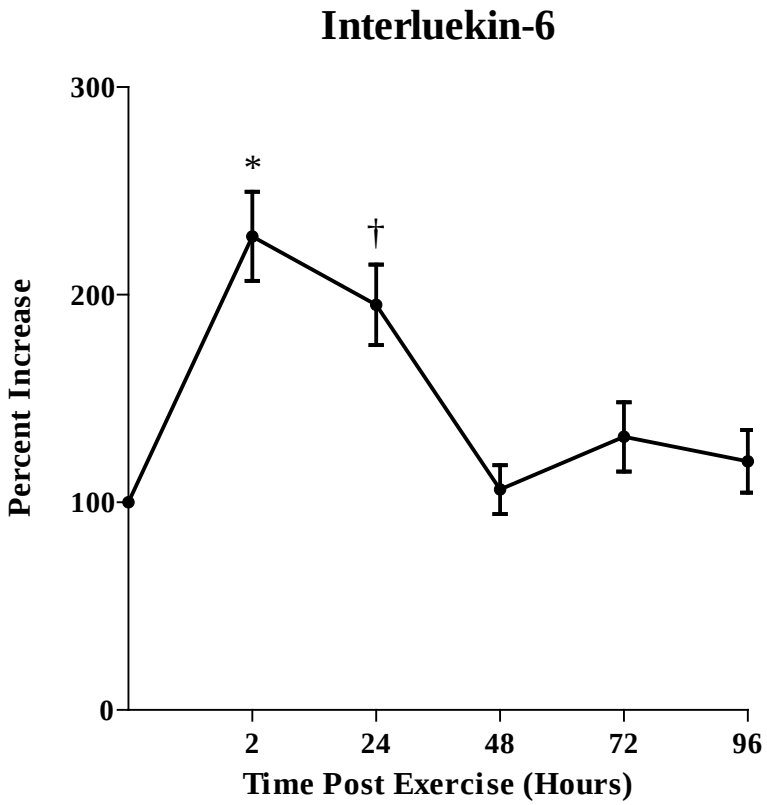
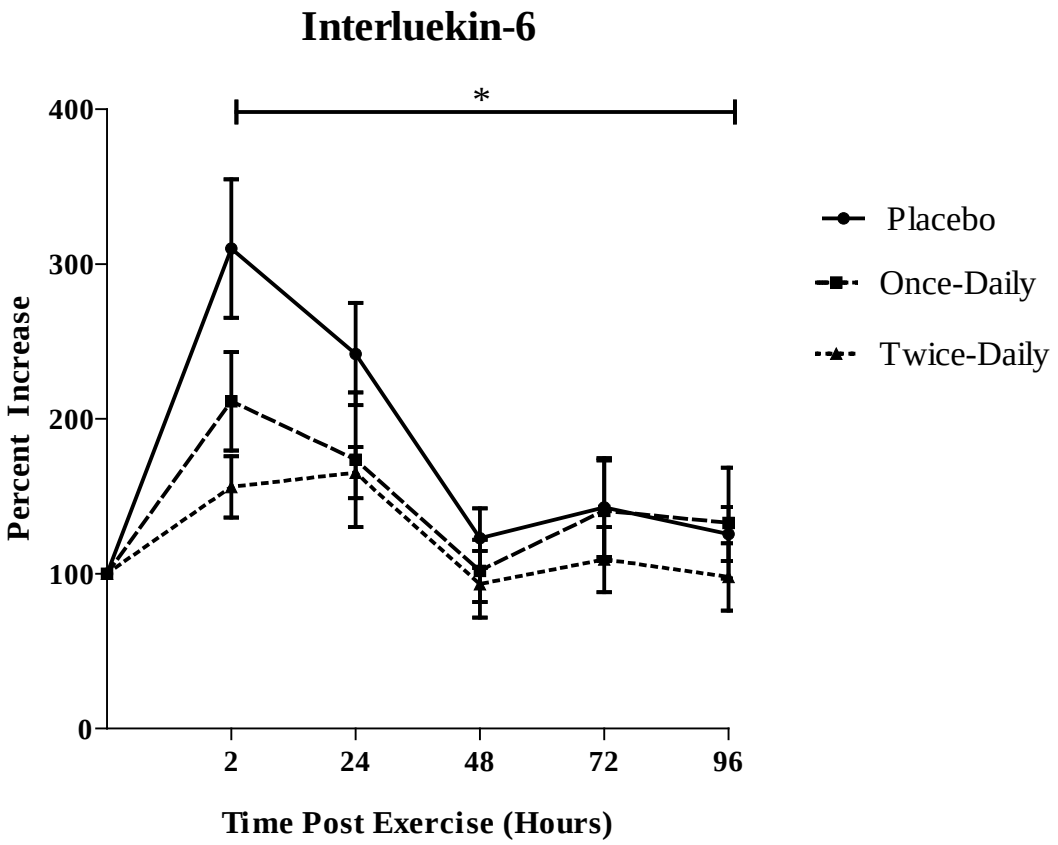

Interluekin-6
Percent Increase
300
200
100
0
*
†
2
24
48
72
96
Time Post Exercise (Hours)

Figure 12


Interluekin-6
*
400
300
200
100
0
Percent Increase
2
24
48
72
96
Time Post Exercise (Hours)
Placebo
Once-Daily
Twice-Daily

DISCUSSION

The major finding in this study is that pomegranate juice supplementation twice a day over eight days attenuated the relative rise of the inflammatory marker IL-6 in the 2-96 h period following an eccentric bout of exercise compared to placebo. The present study suggests that eccentric muscle damage occurred and resulted in increases in CK, Mb, and IL-6. To our knowledge this is the first study to show attenuations in the rise of IL-6 compared to control with twice daily pomegranate juice supplementation following eccentric exercise. This supports our hypothesis that pomegranate juice supplementation might reduce inflammation following eccentric exercise. We were unable to show changes in total anti-oxidant status after exercise with or without supplementation. Other dietary interventions such as vitamin C & E supplementation have been unsuccessful at attenuating the biochemical measures (CK, Mb, and proinflammatory cytokines) after eccentric exercise [31, 63], which is thought to be due to the source or dose of the supplement. For example, most studies do not support the idea that vitamin supplementation reduces muscle damage [63, 68-72], while other non-vitamin anti-inflammatory supplements show beneficial effects following eccentric exercise [7-9, 58, 61].

This study was able to show attenuation in the relative increase in IL-6, likely due to the use of phenolics in the pomegranate concentrate, which have been previously shown to improve strength recovery [7-9] and decreased soreness and pain [7, 9, 11, 12], following eccentric exercise. The findings in this study are similar to other studies utilizing omega-3 fatty acid supplements, which have been able to blunt increases in pro-

inflammatory cytokines and markers of muscle damage after eccentric exercise [58, 62]. However our findings conflict with previous research on pomegranate juice concentrate, which showed no effect of supplementation to attenuate IL-6 following eccentric exercise [8]. The most immediate answer would be mode of eccentric exercise. Supplementation content was the same (as measured by gallic acid equivalent), but the current study incorporated a different eccentric protocol potentially recruiting more muscle because of the downhill running, allowing for the effects of supplementation to be apparent. We propose that twice daily pomegranate juice supplementation could have a beneficial effect, by attenuating the increase in IL-6 during the 2-96 h period following eccentric exercise.

There is a section of the literature that supports the positive role of IL-6 in adaptation to eccentric exercise [42, 44, 46], which would suggest supplementation to be detrimental. Thus, it is unknown whether a supplement that reduces IL-6 would impair or enhance muscle repair and adaption processes after eccentric exercise. While multiple studies do show an improvement in strength recovery with polyphenol supplementation [7-9], it has yet to be determined if supplementation alters the protection conferred due to eccentric exercise.

The ellagitannins found in pomegranate juice have been described as the active constituent in pomegranate juice that would have facilitated changes in IL-6 [83]. In this study, pomegranate juice supplementation did not result in an increase in total anti-oxidant status, but not all studies have found this effect [68]. Our results might be due to a lack of sensitivity in detecting any single anti-oxidant in the blood, especially since the

contributions of endogenous anti-oxidants are major contributor to total anti-oxidant status [10, 73]. Future studies should use more sensitive and specific measures of the anti-oxidant capabilities of pomegranate juice supplementation.

We also were unable to show a decrease in the total anti-oxidant status of the serum after eccentric exercise using total anti-oxidant status. Even in studies using the ratio of reduced glutathione *vs*. oxidized glutathione, a very sensitive measure of the oxidative stress produced during exercise, were unable to show evidence of an oxidative stress response after eccentric exercise [98, 100]. The inconsistency or inability to detect oxidative stress after eccentric exercise is likely due to the degree of muscle damage induced, an inability to detect changes in muscle with plasma measures, or differences in the specific measurements [101]. Our inability to detect changes in total anti-oxidant status following eccentric damage is likely due to one of these factors, most significantly the assay type.

Our data show increases in myoglobin and creatine kinase after exercise, which agrees with the current literature [31, 32, 102]. However, supplementation did not attenuate the rise in either of these intracellular proteins. While most studies show increases with creatine kinase and myoglobin it was very unusual to see such a delayed increase in creatine kinase and two peaks with myoglobin. The 96-hour post exercise peak in creatine kinase and myoglobin may be explained from the mode of exercise (eccentric elbow flexion) as similar protocols have shown peaks occurring at the same time [21, 55, 91]. Alternatively, the early rise in myoglobin might be explained from the downhill running as several studies have shown early peak values of myoglobin within

the first 24 hours following eccentric exercise [25, 31, 32]. This data might suggest that the odd and atypical response we show is due to the two modes of eccentric damage we used during our protocol. Furthermore, we speculated that pomegranate juice supplementation may not have a direct benefit to maintain muscle cell membrane permeability. Many other studies also have shown increases in intracellular proteins regardless of supplementation suggesting no effect of the treatment to attenuate muscle permeability [31, 62, 63]. It is therefore possible that the pomegranate juice provided beneficial effects by reducing inflammation without significantly improving muscle membrane permeability.

Future studies should use more direct measures of muscle inflammation, muscle damage, and redox state by taking muscle biopsies. Without specific measures for muscle damage (i.e.: isometric strength), or soreness we are unable to elaborate on the potential ergogenic or ergolytic effects of the pomegranate juice supplementation. It should also be investigated if supplementation with pomegranate juice alters the protection inferred through repeated bouts of eccentric exercise, which could determine if the inflammation following eccentric exercise plays a role in adaptation. Future studies should elucidate what changes are occurring intracellularly to attenuate the systemic IL-6 response shown in the current study.

In summary we found that pomegranate juice supplementation twice daily for eight days attenuated the relative rise in IL-6 during the 2-96 h period following eccentric muscle damage. We attribute this finding to the ellagitanins found in pomegranate juice, which are known to be anti-inflammatory and anti-oxidants. Future studies should focus

on uncovering the mechanism for attenuating IL-6 and the specific effects of IL-6 this response on overall muscle repair and training responses.

APPENDIX

Biochemical Measurements

Raw Data

Creatine Kinase (CK)

Myoglobin (Mb)

Total-Anti-oxidant Status (TAS)

Interluekin-6 (IL-6)

Interluekin-6 (Fold increase over baseline)

Creatine Kinase (U/L)

Placebo	0	2	24	48	73	96
1	62.79	126.07	170.56	160.34	151.28	457.46
2	132.49	202.69	344.41	230.38	155.73	139.58
5	92.45	139.58	198.41	120.30	92.12	124.09
7	115.19	233.84	542.33	400.44	274.38	399.45
12	98.05	158.86	459.11	185.06	137.44	125.24
18	106.13	155.40	219.67	125.08	244.55	101.18
21	239.61	358.26	766.77	811.60	3473.79	6580.10
24	230.54	561.77	1024.51	844.72	3058.52	3819.03
30	83.05	171.05	319.04	190.33	167.92	167.43
33	156.39	212.58	233.01	226.26	210.27	631.81
36	88.00	122.93	275.53	134.47	277.18	426.97
39	160.67	269.10	339.14	213.57	234.83	223.95
42	164.13	194.95	563.91	329.42	523.38	778.31
45	119.80	239.44	153.09	121.78	197.91	1095.20
50	932.06	825.44	490.25	399.29	374.90	869.93
Mean	**185.42**	**264.80**	**406.65**	**299.54**	**638.28**	**1062.65**
SEM	**54.94**	**49.35**	**62.72**	**60.31**	**277.56**	**460.83**

1x	0	2	24	48	72	96
3	134.30	156.06	285.75	306.84	145.35	214.23
9	76.79	106.13	161.66	259.55	397.31	228.89
11	206.32	242.74	224.28	150.95	116.01	111.56
13	96.40	127.71	321.34	259.38	5619.37	12437.60
17	62.13	148.15	97.06	81.08	94.43	86.84
20	259.88	333.21	258.23	221.64	227.08	234.17
26	38.07	141.89	336.67	247.68	199.56	179.46
29	127.88	159.35	194.62	151.61	135.29	135.62
32	205.82	292.17	541.50	305.03	1461.37	3228.09
35	142.21	182.75	636.75	272.89	157.05	232.19
38	181.76	166.27	333.70	214.56	168.58	159.35
41	89.32	215.88	300.08	178.47	824.94	5710.83
44	273.88	381.33	414.78	395.83	277.18	200.72
48	66.91	222.96	488.77	348.86	247.19	876.36
49	132.16	229.06	538.87	521.56	1597.32	1102.78

Mean	**139.59**	**207.04**	**342.27**	**261.06**	**777.87**	**1675.91**
SEM	**18.68**	**20.35**	**39.81**	**28.04**	**367.56**	**867.19**

2x	**0**	**2**	**24**	**48**	**72**	**96**
4	71.02	168.58	459.44	237.79	134.63	135.13
6	190.00	246.53	426.81	387.09	363.03	351.99
8	224.45	235.98	281.63	298.11	220.33	172.04
10	114.04	153.75	215.55	219.67	174.02	224.94
14	155.40	221.48	333.37	195.61	134.63	388.08
16	116.67	187.37	310.14	365.18	326.62	241.75
19	202.36	298.27	347.87	256.41	268.28	277.34
22	111.56	168.75	295.47	1173.64	4165.09	7197.25
25	241.91	374.24	308.82	351.00	269.60	356.28
28	126.07	205.33	444.44	5144.94	15844.65	30601.69
31	181.60	180.12	225.43	148.81	198.90	185.88
34	90.64	148.64	490.42	516.78	876.85	1503.22
40	171.55	194.29	178.47	184.73	137.44	107.77
43	166.11	240.43	193.63	159.52	143.37	111.07
46	299.26	296.13	1050.71	724.09	1654.17	1617.42

Mean	**164.18**	**221.33**	**370.81**	**690.89**	**1660.77**	**2898.12**
SEM	**15.97**	**16.23**	**54.76**	**325.61**	**1049.06**	**2032.88**

Myoglobin (ng/mL)

Placebo	0	2	24	48	73	96
1	10.28	176.76	40.88	20.83	26.11	140.89
2	8.81	82.65	45.94	8.81	19.78	24.63
5	32.86	116.41	58.81	70.21	44.04	24.00
7	41.51	251.03	74.43	53.33	44.46	37.29
12	13.85	260.52	40.93	40.93	6.57	26.66
18	19.98	116.13	25.51	34.63	48.99	33.25
21	29.10	140.72	60.60	87.40	372.24	484.97
24	17.59	387.90	35.43	76.43	157.62	408.93
30	19.72	211.40	26.11	41.82	53.80	93.47
33	26.09	154.21	29.04	39.49	17.25	39.76
36	10.00	30.65	30.77	16.83	25.29	60.94
39	27.17	73.27	15.64	11.35	22.34	3.04
42	3.63	196.52	22.50	29.77	22.70	34.39
45	24.72	32.13	2.85	2.55	25.51	23.14
50	37.08	105.45	21.04	23.41	33.14	57.33
Mean	**21.49**	**155.72**	**35.37**	**37.19**	**61.32**	**99.51**
SEM	**2.85**	**24.70**	**4.85**	**6.57**	**24.01**	**37.62**

1x	0	2	24	48	73	96
3	10.07	151.44	38.13	20.62	20.20	19.78
9	23.75	45.01	31.61	96.85	35.69	30.74
11	39.18	194.12	38.02	25.21	24.62	12.39
13	26.08	171.40	35.78	138.86	715.25	626.90
17	16.39	75.79	20.54	26.34	18.88	17.50
20	62.81	138.23	50.65	33.80	71.93	63.08
26	22.92	355.69	79.09	22.92	40.49	29.04
29	16.00	82.55	29.84	33.04	16.00	12.00
32	15.91	261.15	22.88	16.18	318.24	275.35
35	9.48	227.11	62.81	22.07	18.59	13.50
38	10.55	46.46	28.51	5.72	14.03	7.06
41	11.31	94.43	13.46	21.62	227.27	969.51
44	7.56	89.42	21.12	24.76	7.76	13.07
48	32.08	406.27	48.12	56.01	115.18	220.62
49	37.08	336.06	39.97	183.81	132.80	78.63

Mean	**22.75**	**178.34**	**37.37**	**48.52**	**118.46**	**159.28**
SEM	**3.88**	**30.08**	**4.47**	**13.21**	**48.56**	**72.05**

2x	**0**	**2**	**24**	**48**	**73**	**96**
4	32.65	30.75	67.25	33.28	44.89	51.64
6	13.66	114.73	29.06	28.01	35.39	23.36
8	5.40	137.62	12.10	19.09	24.33	22.29
10	22.00	143.15	42.97	14.72	10.35	30.74
14	21.42	168.78	13.56	17.92	14.72	53.45
16	29.65	90.71	43.47	34.07	46.51	30.48
19	57.01	205.65	72.20	65.85	51.76	86.02
22	39.05	48.72	75.75	402.86	1463.08	1559.85
25	38.36	154.96	57.26	55.66	32.50	69.51
28	24.52	233.76	142.98	1112.29	1339.11	690.13
31	29.57	82.55	11.47	21.59	16.53	33.57
34	13.23	51.29	58.26	41.91	101.68	254.45
40	5.99	47.16	8.15	10.61	12.28	9.63
43	30.77	37.24	5.40	11.10	30.24	10.02
46	3.14	124.69	25.45	25.15	97.37	64.46

Mean	**24.43**	**111.45**	**44.36**	**126.27**	**221.38**	**199.31**
SEM	**3.81**	**16.31**	**9.48**	**74.79**	**124.02**	**107.00**

Total Anti-oxidant Status (mM trolox equivalents)

Placebo	Pre-Supp	0	24
1	1.97	2.03	1.95
2	1.94	2.06	1.96
5	1.93	1.93	2.13
7	1.81	1.96	1.83
12	1.93	2.00	1.98
18	1.78	2.09	1.96
21	2.08	1.79	1.77
24	1.69	1.53	1.54
30	1.40	1.19	1.36
33	0.97	1.16	1.23
36	0.56	0.60	0.60
39	1.88	1.17	1.32
42	0.71	0.50	1.51
45	1.11	1.59	0.60
50	0.63	1.00	1.19

Mean	**1.49**	**1.51**	**1.53**
SEM	**0.14**	**0.14**	**0.12**

1x	Pre-Supp	0	24
3	2.00	2.07	1.88
9	1.86	1.98	1.67
11	1.94	1.99	1.98
13	1.99	2.00	1.96
17	2.05	1.90	2.03
20	2.11	1.97	2.01
26	1.33	1.73	1.82
29	1.61	1.64	1.90
32	0.68	0.78	1.43
35	1.13	0.88	1.48
38	1.66	1.53	1.47
41	0.96	1.12	0.60
44	1.33	1.48	0.77
48	0.65	0.84	0.83
49	0.45	0.41	0.75

Mean	**1.45**	**1.49**	**1.51**
SEM	**0.15**	**0.14**	**0.13**

2x	**Pre-Supp**	**0**	**24**
4	1.95	1.99	2.08
6	2.18	2.10	1.96
8	1.88	1.91	1.97
10	1.95	1.94	1.97
14	2.05	1.93	1.92
16	2.02	2.03	1.83
19	1.93	1.92	2.31
22	1.74	1.49	1.77
25	1.57	1.75	1.57
28	1.25	1.72	1.58
31	1.62	1.67	1.00
34	1.17	0.80	1.63
40	0.99	1.03	0.82
43	0.86	0.73	1.04
46	1.06	1.34	1.05

Mean	**1.62**	**1.62**	**1.63**
SEM	**0.11**	**0.12**	**0.12**

Interluekin-6 (pg/mL)

Placebo	**0**	**2**	**24**	**48**	**73**	**96**
1	3.93	5.92	3.93	4.93	2.93	1.93
2	2.93	3.93	5.92	1.93	3.93	3.93
5	3.93	7.92	10.91	4.93	3.93	2.93
7	2.29	5.34	6.36	4.32	1.27	1.27
12	2.29	5.34	6.36	3.31	2.29	3.31
18	1.80	7.82	6.10	1.80	3.52	4.38
21	1.07	6.03	3.05	0.07	4.05	1.07
24	1.07	5.04	3.05	3.05	0.07	3.05
30	1.41	9.03	8.08	3.31	6.17	2.36
33	2.36	6.17	8.08	4.27	4.27	2.36
36	1.32	7.27	1.32	1.32	1.32	1.98
39	2.64	5.94	2.64	2.64	2.64	1.32
42	3.37	4.09	6.97	1.21	6.97	3.37
45	3.37	6.97	3.37	2.65	1.93	3.37
50	2.46	5.35	3.91	1.74	0.30	3.19
Mean	**2.41**	**6.14**	**5.34**	**2.76**	**3.04**	**2.65**
SEM	**0.25**	**0.37**	**0.67**	**0.37**	**0.51**	**0.26**

1x	**0**	**2**	**24**	**48**	**72**	**96**
3	3.93	4.93	4.93	3.93	3.93	2.93
9	4.32	7.37	7.37	4.32	3.31	2.29
11	2.29	5.34	5.34	3.31	1.27	4.32
13	3.52	4.38	5.24	0.94	0.94	0.94
17	1.80	4.38	2.66	1.80	1.80	0.94
20	2.06	2.06	1.07	1.07	4.44	1.07
26	1.07	4.05	0.07	0.07	5.04	2.06
29	2.36	5.22	12.84	8.08	9.03	13.80
32	1.41	8.08	1.41	1.41	1.41	2.36
35	1.98	4.62	1.32	2.64	2.64	3.30
38	2.64	3.96	15.20	2.64	3.96	2.64
41	3.37	4.09	6.97	2.65	3.37	3.37
44	4.09	7.69	3.37	4.81	3.37	1.93
48	2.46	4.63	2.46	2.46	1.74	3.91
49	3.91	4.63	1.74	1.02	1.74	1.02

Mean	**2.75**	**5.03**	**4.80**	**2.74**	**3.20**	**3.12**
SEM	**0.27**	**0.41**	**1.13**	**0.52**	**0.53**	**0.81**

2x	**0**	**2**	**24**	**48**	**72**	**96**
4	5.92	6.92	6.92	4.93	2.93	2.93
6	5.92	4.93	7.92	3.93	0.93	4.93
8	3.31	7.37	5.34	3.31	1.27	1.27
10	4.32	3.31	6.36	1.27	6.36	3.31
14	3.52	2.66	8.68	0.08	0.94	3.52
16	3.52	7.82	4.38	1.80	2.66	0.94
19	3.52	3.52	6.10	0.08	8.68	4.38
22	3.05	3.05	2.06	4.05	2.06	2.06
25	2.06	3.05	4.05	0.07	1.07	1.07
28	2.36	4.27	6.17	7.13	6.17	5.22
31	4.27	10.94	9.03	4.27	3.31	4.27
34	2.64	8.59	6.61	5.94	5.28	1.32
40	1.32	2.64	2.64	1.98	2.64	4.62
43	3.37	2.65	4.81	2.65	2.65	1.93
46	2.65	4.09	1.21	1.93	2.65	1.93

Mean	**3.45**	**5.05**	**5.48**	**2.89**	**3.31**	**2.91**
SEM	**0.33**	**0.68**	**0.60**	**0.56**	**0.59**	**0.39**

Interluekin-6 (percent increase of baseline)

Placebo	**0**	**2**	**24**	**48**	**72**	**96**
1	100.00	150.82	100.00	125.41	74.59	49.18
2	100.00	134.07	202.20	65.93	134.07	134.07
5	100.00	201.64	277.87	125.41	100.00	74.59
7	100.00	233.13	277.51	188.75	55.62	55.62
12	100.00	233.13	277.51	144.38	100.00	144.38
18	100.00	435.32	339.51	100.00	195.81	243.71
21	100.00	565.67	286.27	6.87	379.40	100.00
24	100.00	472.53	286.27	286.27	6.87	286.27
30	100.00	641.77	574.05	235.44	438.61	167.72
33	100.00	261.51	342.26	180.75	180.75	100.00
36	100.00	551.04	100.00	100.00	100.00	150.12
39	100.00	225.14	100.00	100.00	100.00	49.94
42	100.00	121.38	206.91	35.85	206.91	100.00
45	100.00	206.91	100.00	78.62	57.23	100.00
50	100.00	217.30	158.65	70.68	12.03	129.32

Mean	**100.00**	**310.09**	**241.93**	**122.96**	**142.79**	**125.66**
SEM	**0.00**	**44.71**	**32.95**	**19.28**	**31.95**	**17.49**

1x	**0**	**2**	**24**	**48**	**72**	**96**
3	100.00	125.41	125.41	100.00	100.00	74.59
9	100.00	170.53	170.53	100.00	76.49	52.98
11	100.00	233.13	233.13	144.38	55.62	188.75
13	100.00	124.46	148.93	26.61	26.61	26.61
17	100.00	243.71	147.90	100.00	100.00	52.10
20	100.00	100.00	51.78	51.78	215.73	51.78
26	100.00	379.40	6.87	6.87	472.53	193.13
29	100.00	221.13	544.15	342.26	382.64	584.53
32	100.00	574.05	100.00	100.00	100.00	167.72
35	100.00	233.54	66.62	133.38	133.38	166.77
38	100.00	150.06	575.55	100.00	150.06	100.00
41	100.00	121.38	206.91	78.62	100.00	100.00
44	100.00	188.08	82.38	117.62	82.38	47.15
48	100.00	187.97	100.00	100.00	70.68	158.65
49	100.00	118.48	44.55	26.06	44.55	26.06

Mean	**100.00**	**211.42**	**173.65**	**101.84**	**140.71**	**132.72**
SEM	**0.00**	**31.81**	**43.48**	**20.04**	**32.58**	**35.78**

2x	**0**	**2**	**24**	**48**	**72**	**96**
4	100.00	116.85	116.85	83.15	49.46	49.46
6	100.00	83.15	133.70	66.30	15.76	83.15
8	100.00	222.95	161.47	100.00	38.53	38.53
10	100.00	76.49	147.02	29.47	147.02	76.49
14	100.00	75.54	246.79	2.14	26.61	100.00
16	100.00	222.32	124.46	51.07	75.54	26.61
19	100.00	100.00	173.39	2.14	246.79	124.46
22	100.00	100.00	67.47	132.53	67.47	67.47
25	100.00	148.22	196.44	3.56	51.78	51.78
28	100.00	180.75	261.51	301.89	261.51	221.13
31	100.00	256.37	211.69	100.00	77.66	100.00
34	100.00	325.26	250.17	225.14	200.12	49.94
40	100.00	200.23	200.23	150.12	200.23	350.58
43	100.00	78.62	142.77	78.62	78.62	57.23
46	100.00	154.40	45.60	72.80	100.00	72.80

Mean	**100.00**	**156.08**	**165.30**	**93.26**	**109.14**	**97.98**
SEM	**0.00**	**19.78**	**16.52**	**21.57**	**20.98**	**21.71**

REFRENCES

1. Morgan, D.L. and U. Proske, *Popping sarcomere hypothesis explains stretch-induced muscle damage.* Clin Exp Pharmacol Physiol, 2004. **31**(8): p. 541-5.
2. Rodenburg, J.B., P.R. Bar, and R.W. De Boer, *Relations between muscle soreness and biochemical and functional outcomes of eccentric exercise.* J Appl Physiol, 1993. **74**(6): p. 2976-2983.
3. MacIntyre, D.L., et al., *Presence of WBC, decreased strength, and delayed soreness in muscle after eccentric exercise.* J Appl Physiol, 1996. **80**(3): p. 1006-1013.
4. Leeuwenburgh, C. and J.W. Heinecke, *Oxidative stress and antioxidants in exercise.* Curr Med Chem, 2001. **8**(7): p. 829-38.
5. Raastad, T., et al., *Temporal relation between leukocyte accumulation in muscles and halted recovery 10-20 h after strength exercise.* J Appl Physiol, 2003. **95**(6): p. 2503-9.
6. MacIntyre, D.L., et al., *Presence of WBC, decreased strength, and delayed soreness in muscle after eccentric exercise.* J Appl Physiol, 1996. **80**(3): p. 1006-13.
7. Connolly, D.A., et al., *Efficacy of a tart cherry juice blend in preventing the symptoms of muscle damage.* Br J Sports Med, 2006. **40**(8): p. 679-83; discussion 683.
8. Trombold, J.R., et al., *Ellagitannin consumption improves strength recovery 2-3 d after eccentric exercise.* Med Sci Sports Exerc, 2010. **42**(3): p. 493-8.
9. Trombold, J.R., et al., *The effect of pomegranate juice supplementation on strength and soreness after eccentric exercise.* J Strength Cond Res, 2011. **25**(7): p. 1782-8.
10. Collins, A.R., *Assays for oxidative stress and antioxidant status: applications to research into the biological effectiveness of polyphenols.* Am J Clin Nutr, 2005. **81**(1 Suppl): p. 261S-267S.
11. Howatson, G., et al., *Influence of tart cherry juice on indices of recovery following marathon running.* Scand J Med Sci Sports, 2010. **20**(6): p. 843-52.
12. Kuehl, K.S., et al., *Efficacy of tart cherry juice in reducing muscle pain during running: a randomized controlled trial.* J Int Soc Sports Nutr, 2010. **7**: p. 17.
13. Armstrong, R.B., R.W. Ogilvie, and J.A. Schwane, *Eccentric exercise-induced injury to rat skeletal muscle.* J Appl Physiol, 1983. **54**(1): p. 80-93.
14. Friden, J., M. Sjostrom, and B. Ekblom, *Myofibrillar damage following intense eccentric exercise in man.* Int J Sports Med, 1983. **4**(3): p. 170-6.
15. Newham, D.J., et al., *Pain and fatigue after concentric and eccentric muscle contractions.* Clin Sci (Lond), 1983. **64**(1): p. 55-62.
16. Friden, J., et al., *Adaptive response in human skeletal muscle subjected to prolonged eccentric training.* Int J Sports Med, 1983. **4**(3): p. 177-83.
17. Morgan, D.L., *New insights into the behavior of muscle during active lengthening.* Biophys J, 1990. **57**(2): p. 209-21.

18. Brockett, C.L., et al., *Damage to different motor units from active lengthening of the medial gastrocnemius muscle of the cat.* J Appl Physiol, 2002. **92**(3): p. 1104-10.
19. Lynn, R. and D.L. Morgan, *Decline running produces more sarcomeres in rat vastus intermedius muscle fibers than does incline running.* J Appl Physiol, 1994. **77**(3): p. 1439-44.
20. Lynn, R., J.A. Talbot, and D.L. Morgan, *Differences in rat skeletal muscles after incline and decline running.* J Appl Physiol, 1998. **85**(1): p. 98-104.
21. Chen, T.C. and K. Nosaka, *Responses of elbow flexors to two strenuous eccentric exercise bouts separated by three days.* J Strength Cond Res, 2006. **20**(1): p. 108-16.
22. Chen, T.C., K. Nosaka, and P. Sacco, *Intensity of eccentric exercise, shift of optimum angle, and the magnitude of repeated-bout effect.* J Appl Physiol, 2007. **102**(3): p. 992-999.
23. Warren, G.L., D.A. Lowe, and R.B. Armstrong, *Measurement tools used in the study of eccentric contraction-induced injury.* Sports Med, 1999. **27**(1): p. 43-59.
24. Jones, D.A., D.J. Newham, and C. Torgan, *Mechanical influences on long-lasting human muscle fatigue and delayed-onset pain.* J Physiol, 1989. **412**: p. 415-27.
25. Byrnes, W.C., et al., *Delayed onset muscle soreness following repeated bouts of downhill running.* J Appl Physiol, 1985. **59**(3): p. 710-715.
26. Clarkson, P.M., et al., *Muscle soreness and serum creatine kinase activity following isometric, eccentric, and concentric exercise.* Int J Sports Med, 1986. **7**(3): p. 152-5.
27. Newham, D.J., D.A. Jones, and P.M. Clarkson, *Repeated high-force eccentric exercise: effects on muscle pain and damage.* J Appl Physiol, 1987. **63**(4): p. 1381-1386.
28. Eston, R.G., et al., *Muscle tenderness and peak torque changes after downhill running following a prior bout of isokinetic eccentric exercise.* J Sports Sci, 1996. **14**(4): p. 291-9.
29. Lieber, R.L. and J. Friden, *Muscle damage is not a function of muscle force but active muscle strain.* J Appl Physiol, 1993. **74**(2): p. 520-6.
30. Armstrong, R.B., *Initial events in exercise-induced muscular injury.* Med Sci Sports Exerc, 1990. **22**(4): p. 429-35.
31. Kingsley, M.I., et al., *Phosphatidylserine supplementation and recovery following downhill running.* Med Sci Sports Exerc, 2006. **38**(9): p. 1617-25.
32. Peake, J.M., et al., *Exercise-induced muscle damage, plasma cytokines, and markers of neutrophil activation.* Med Sci Sports Exerc, 2005. **37**(5): p. 737-45.
33. Teague, B.N. and J.A. Schwane, *Effect of intermittent eccentric contractions on symptoms of muscle microinjury.* Med Sci Sports Exerc, 1995. **27**(10): p. 1378-84.
34. MacIntyre, D.L., W.D. Reid, and D.C. McKenzie, *Delayed muscle soreness. The inflammatory response to muscle injury and its clinical implications.* Sports Med, 1995. **20**(1): p. 24-40.

35. Belcastro, A.N., L.D. Shewchuk, and D.A. Raj, *Exercise-induced muscle injury: a calpain hypothesis.* Mol Cell Biochem, 1998. **179**(1-2): p. 135-45.
36. MacIntyre, D.L., et al., *Markers of inflammation and myofibrillar proteins following eccentric exercise in humans.* Eur J Appl Physiol, 2001. **84**(3): p. 180-6.
37. Beaton, L.J., M.A. Tarnopolsky, and S.M. Phillips, *Contraction-induced muscle damage in humans following calcium channel blocker administration.* J Physiol, 2002. **544**(Pt 3): p. 849-59.
38. Jones, D.A., et al., *Experimental human muscle damage: morphological changes in relation to other indices of damage.* J Physiol, 1986. **375**: p. 435-48.
39. Nguyen, H.X. and J.G. Tidball, *Null mutation of gp91phox reduces muscle membrane lysis during muscle inflammation in mice.* J Physiol, 2003. **553**(Pt 3): p. 833-41.
40. Nguyen, H.X. and J.G. Tidball, *Interactions between neutrophils and macrophages promote macrophage killing of rat muscle cells in vitro.* J Physiol, 2003. **547**(Pt 1): p. 125-32.
41. Peake, J., K. Nosaka, and K. Suzuki, *Characterization of inflammatory responses to eccentric exercise in humans.* Exerc Immunol Rev, 2005. **11**: p. 64-85.
42. Koh, T.J. and S.V. Brooks, *Lengthening contractions are not required to induce protection from contraction-induced muscle injury.* Am J Physiol Regul Integr Comp Physiol, 2001. **281**(1): p. R155-61.
43. Ochi, E., K. Nakazato, and N. Ishii, *Muscular hypertrophy and changes in cytokine production after eccentric training in the rat skeletal muscle.* J Strength Cond Res, 2011. **25**(8): p. 2283-92.
44. Pizza, F.X., et al., *Muscle inflammatory cells after passive stretches, isometric contractions, and lengthening contractions.* J Appl Physiol, 2002. **92**(5): p. 1873-8.
45. Serrano, A.L., et al., *Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy.* Cell Metab, 2008. **7**(1): p. 33-44.
46. Chen, T.C. and S.S. Hsieh, *Effects of a 7-day eccentric training period on muscle damage and inflammation.* Med Sci Sports Exerc, 2001. **33**(10): p. 1732-8.
47. Halliwell, B., *Free radicals and antioxidants: a personal view.* Nutr Rev, 1994. **52**(8 Pt 1): p. 253-65.
48. Cheeseman, K.H. and T.F. Slater, *An introduction to free radical biochemistry.* Br Med Bull, 1993. **49**(3): p. 481-93.
49. Davies, K.J., et al., *Free radicals and tissue damage produced by exercise.* Biochem Biophys Res Commun, 1982. **107**(4): p. 1198-205.
50. McBride, J.M. and W.J. Kraemer, *Free Radicals, Exercise, and Antioxidants.* The Journal of Strength & Conditioning Research, 1999. **13**(2): p. 175-183.
51. Radák, Z., *Free radicals in exercise and aging*. 2000: Human Kinetics.
52. Malm, C., R. Lenkei, and B. Sjodin, *Effects of eccentric exercise on the immune system in men.* J Appl Physiol, 1999. **86**(2): p. 461-8.
53. Schwane, J.A. and R.B. Armstrong, *Effect of training on skeletal muscle injury from downhill running in rats.* J Appl Physiol, 1983. **55**(3): p. 969-75.

54. Clarkson, P.M., et al., *Serum creatine kinase activity following forearm flexion isometric exercise.* Eur J Appl Physiol Occup Physiol, 1985. **53**(4): p. 368-71.
55. Chen, T.C., K. Nosaka, and P. Sacco, *Intensity of eccentric exercise, shift of optimum angle, and the magnitude of repeated-bout effect.* J Appl Physiol, 2007. **102**(3): p. 992-9.
56. Chen, T.C., et al., *Potent protective effect conferred by four bouts of low-intensity eccentric exercise.* Med Sci Sports Exerc, 2010. **42**(5): p. 1004-12.
57. Chen, C.H., et al., *Effects of flexibility training on eccentric exercise-induced muscle damage.* Med Sci Sports Exerc, 2011. **43**(3): p. 491-500.
58. Tartibian, B., B.H. Maleki, and A. Abbasi, *Omega-3 fatty acids supplementation attenuates inflammatory markers after eccentric exercise in untrained men.* Clin J Sport Med, 2011. **21**(2): p. 131-7.
59. Vincent, B., et al., *Protective role of alpha-actinin-3 in the response to an acute eccentric exercise bout.* J Appl Physiol, 2010. **109**(2): p. 564-73.
60. Beavers, K.M., et al., *Soy and the exercise-induced inflammatory response in postmenopausal women.* Appl Physiol Nutr Metab, 2010. **35**(3): p. 261-9.
61. Davis, J.M., et al., *Curcumin effects on inflammation and performance recovery following eccentric exercise-induced muscle damage.* Am J Physiol Regul Integr Comp Physiol, 2007. **292**(6): p. R2168-73.
62. Phillips, T., et al., *A dietary supplement attenuates IL-6 and CRP after eccentric exercise in untrained males.* Med Sci Sports Exerc, 2003. **35**(12): p. 2032-7.
63. Petersen, E.W., et al., *Effect of vitamin supplementation on cytokine response and on muscle damage after strenuous exercise.* Am J Physiol Cell Physiol, 2001. **280**(6): p. C1570-5.
64. Silva, L.A., et al., *N-acetylcysteine supplementation and oxidative damage and inflammatory response after eccentric exercise.* Int J Sport Nutr Exerc Metab, 2008. **18**(4): p. 379-88.
65. Bryer, S.C. and A.H. Goldfarb, *Effect of high dose vitamin C supplementation on muscle soreness, damage, function, and oxidative stress to eccentric exercise.* Int J Sport Nutr Exerc Metab, 2006. **16**(3): p. 270-80.
66. Thompson, D., et al., *Prolonged vitamin C supplementation and recovery from eccentric exercise.* Eur J Appl Physiol, 2004. **92**(1-2): p. 133-8.
67. Van Der Meulen, J.H., et al., *Contraction-induced injury to the extensor digitorum longus muscles of rats: the role of vitamin E.* J Appl Physiol, 1997. **83**(3): p. 817-823.
68. Childs, A., et al., *Supplementation with vitamin C and N-acetyl-cysteine increases oxidative stress in humans after an acute muscle injury induced by eccentric exercise.* Free Radic Biol Med, 2001. **31**(6): p. 745-53.
69. McGinley, C., A. Shafat, and A.E. Donnelly, *Does antioxidant vitamin supplementation protect against muscle damage?* Sports Med, 2009. **39**(12): p. 1011-32.
70. Beaton, L.J., et al., *Contraction-induced muscle damage is unaffected by vitamin E supplementation.* Med Sci Sports Exerc, 2002. **34**(5): p. 798-805.

71. Maxwell, S.R., et al., *Changes in plasma antioxidant status during eccentric exercise and the effect of vitamin supplementation.* Free Radic Res Commun, 1993. **19**(3): p. 191-202.
72. Theodorou, A.A., et al., *No effect of antioxidant supplementation on muscle performance and blood redox status adaptations to eccentric training.* The American journal of clinical nutrition, 2011. **93**(6): p. 1373-83.
73. Griffiths, H.R., et al., *Biomarkers.* Mol Aspects Med, 2002. **23**(1-3): p. 101-208.
74. Thompson, D., et al., *Post-exercise vitamin C supplementation and recovery from demanding exercise.* Eur J Appl Physiol, 2003. **89**(3-4): p. 393-400.
75. Thompson, D., et al., *Muscle soreness and damage parameters after prolonged intermittent shuttle-running following acute vitamin C supplementation.* Int J Sports Med, 2001. **22**(1): p. 68-75.
76. Avery, N.G., et al., *Effects of vitamin E supplementation on recovery from repeated bouts of resistance exercise.* J Strength Cond Res, 2003. **17**(4): p. 801-9.
77. Cannon, J.G., et al., *Acute phase response in exercise. II. Associations between vitamin E, cytokines, and muscle proteolysis.* Am J Physiol, 1991. **260**(6 Pt 2): p. R1235-40.
78. Cannon, J.G., et al., *Acute phase response in exercise: interaction of age and vitamin E on neutrophils and muscle enzyme release.* Am J Physiol, 1990. **259**(6 Pt 2): p. R1214-9.
79. Sacheck, J.M., et al., *Effect of vitamin E and eccentric exercise on selected biomarkers of oxidative stress in young and elderly men.* Free Radic Biol Med, 2003. **34**(12): p. 1575-88.
80. Mastaloudis, A., et al., *Antioxidants did not prevent muscle damage in response to an ultramarathon run.* Med Sci Sports Exerc, 2006. **38**(1): p. 72-80.
81. Davison, G.W., C.M. Hughes, and R.A. Bell, *Exercise and mononuclear cell DNA damage: the effects of antioxidant supplementation.* Int J Sport Nutr Exerc Metab, 2005. **15**(5): p. 480-92.
82. Connolly, D.A.J., et al., *Efficacy of a tart cherry juice blend in preventing the symptoms of muscle damage * Commentary 1 * Commentary 2.* Br. J. Sports Med., 2006. **40**(8): p. 679-683.
83. Adams, L.S., et al., *Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells.* Journal of agricultural and food chemistry, 2006. **54**(3): p. 980-5.
84. Rozenberg, O., A. Howell, and M. Aviram, *Pomegranate juice sugar fraction reduces macrophage oxidative state, whereas white grape juice sugar fraction increases it.* Atherosclerosis, 2006. **188**(1): p. 68-76.
85. Seeram, N.P., et al., *In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice.* J Nutr Biochem, 2005. **16**(6): p. 360-7.
86. Faria, A., et al., *Effect of pomegranate (Punica granatum) juice intake on hepatic oxidative stress.* Eur J Nutr, 2007. **46**(5): p. 271-8.

87. Gil, M.I., et al., *Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing.* J Agric Food Chem, 2000. **48**(10): p. 4581-9.
88. Seeram, N.P., et al., *Rapid large scale purification of ellagitannins from pomegranate husk, a by-product of the commercial juice industry*. 2005.
89. Clarkson, P.M. and I. Tremblay, *Exercise-induced muscle damage, repair, and adaptation in humans.* J Appl Physiol, 1988. **65**(1): p. 1-6.
90. Gunst, J.J., et al., *Serum creatine kinase activity is not a reliable marker for muscle damage in conditions associated with low extracellular glutathione concentration.* Clin Chem, 1998. **44**(5): p. 939-43.
91. Lee, J. and P.M. Clarkson, *Plasma creatine kinase activity and glutathione after eccentric exercise.* Med Sci Sports Exerc, 2003. **35**(6): p. 930-6.
92. Willoughby, D.S., B. McFarlin, and C. Bois, *Interleukin-6 expression after repeated bouts of eccentric exercise.* Int J Sports Med, 2003. **24**(1): p. 15-21.
93. Tomiya, A., et al., *Myofibers express IL-6 after eccentric exercise.* Am J Sports Med, 2004. **32**(2): p. 503-8.
94. Malm, C., et al., *Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies.* J Physiol, 2000. **529 Pt 1**: p. 243-62.
95. Close, G.L., et al., *Eccentric exercise, isokinetic muscle torque and delayed onset muscle soreness: the role of reactive oxygen species.* Eur J Appl Physiol, 2004. **91**(5-6): p. 615-21.
96. Close, G.L., et al., *Effects of dietary carbohydrate on delayed onset muscle soreness and reactive oxygen species after contraction induced muscle damage.* Br J Sports Med, 2005. **39**(12): p. 948-53.
97. Goldfarb, A.H., R.J. Bloomer, and M.J. McKenzie, *Combined antioxidant treatment effects on blood oxidative stress after eccentric exercise.* Med Sci Sports Exerc, 2005. **37**(2): p. 234-9.
98. Lee, J., et al., *Eccentric exercise effect on blood oxidative-stress markers and delayed onset of muscle soreness.* Med Sci Sports Exerc, 2002. **34**(3): p. 443-8.
99. Bartosz, G., *Total antioxidant capacity.* Adv Clin Chem, 2003. **37**: p. 219-92.
100. Camus, G., et al., *Blood levels of reduced/oxidized glutathione and plasma concentration of ascorbic acid during eccentric and concentric exercises of similar energy cost.* Arch Int Physiol Biochim Biophys, 1994. **102**(1): p. 67-70.
101. Nikolaidis, M.G., et al., *The effect of muscle-damaging exercise on blood and skeletal muscle oxidative stress: magnitude and time-course considerations.* Sports Med, 2008. **38**(7): p. 579-606.
102. Toft, A.D., et al., *Cytokine response to eccentric exercise in young and elderly humans.* Am J Physiol Cell Physiol, 2002. **283**(1): p. C289-95.